Structural Aspects of Platinum Anticancer Drug Interactions with DNA

SUZANNE E. SHERMAN and STEPHEN J. LIPPARD*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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Contents

I. Introduction

The classic coordination complex *cis*-diamminedichloroplatinum(II),[1] *cis*-DDP or cisplatin, has been the subject of much recent attention because of its beneficial effects in the treatment of cancer.[2,3] Some details of the mechanism of action of this antitumor drug are currently understood. A large body of evidence indicates that *cis*-DDP exhibits its biological activity by binding to DNA and inhibiting replication. Furthermore, the characteristic modes by which *cis*-DDP binds to DNA in vitro and in vivo have been defined. Information about which of these binding modes are responsible for activity, and the mechanism by which DNA replication is inhibited in tumor cells, is only beginning to emerge. Knowledge of the structural perturbations that accompany *cis*-DDP reactions with DNA may eventually provide clues to these and other questions. Here, we summarize the results of recent experiments that reveal structural aspects of platinum anticancer drug binding to DNA.

The paper is divided into six parts. The next, or second, section briefly recounts the discovery of anticancer activity for *cis*-DDP and summarizes its clinical applications. Pharmacological aspects are reviewed elsewhere.[4,5] In the third section, we present some of the evidence implicating DNA as the cellular target. More detailed discussions of this topic are available.[6,7] The next two sections treat structural aspects of the interaction of *cis*-DDP with DNA. Included in section IV is a brief description of DNA stereochemistry and nomenclature. More information on these subjects can be obtained from ref 8 and 9. A major portion of section IV discusses different platinum binding modes and their biological consequences, additional details of which are reviewed elsewhere.[10] The present focus is on bifunctional, intrastrand binding modes, about which the most information is available. Section IV also discusses the influence of local DNA structure on platinum binding, as revealed by both biophysical and immunological techniques. In section V are presented the structural details of platinum–DNA adducts, as revealed by NMR spectroscopic and X-ray crystallographic studies. Considerable structural details are known about platinum complexes of DNA constituents, specifically the purine and pyrimidine bases, nucleosides, and nucleotides, which have been examined as models of platinum–DNA interactions.[11–13] Although these data have contributed significantly to an understanding of platinum binding specificity and kinetics,[14] in most cases they have been unable to model accurately the bifunctional, intrastrand cross-linking of DNA nucleosides by *cis*-DDP, since they lack constraints imposed by a DNA sugar–phosphate backbone. These model studies are therefore largely excluded from the scope of this paper. Included are all platinum–oligonucleotide complexes studied thus far that contain bifunctional platinum adducts that form when *cis*-DDP is allowed to react with random-sequence DNA. Results for the chemotherapeutically inactive isomer *trans*-DDP are also included for comparison purposes. Finally, in section VI are discussed some future challenges.

II. History and Clinical Importance of Platinum Anticancer Drugs

In the early 1960s, during a study of the effects of electric fields on bacterial growth, a curious phenomenon was observed. When an electric field was applied

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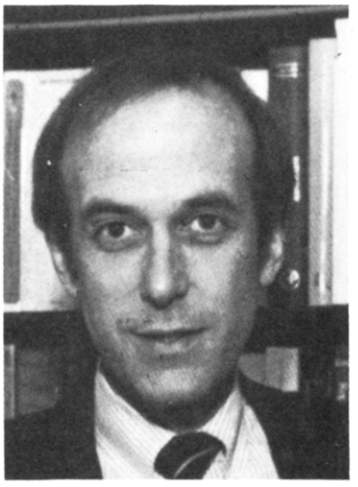
Suzanne E. Sherman, a native of Clarence, NY, received her Bachelor of Science degree (summa cum laude) at the State University of New York at Albany in 1982, where she served as a research assistant to Professor Kenneth D. Karlin. At Albany, she was the recipient of the Derk V. Tieszen award for demonstrated achievement in chemistry, physics, and mathematics. She received her Ph.D. degree under Professor Stephen J. Lippard at the Massachusetts Institute of Technology in May 1987. Her research there included X-ray structural elucidation of the adduct formed between *cis*-diamminedichloroplatinum(II) and the DNA fragment d(pGpG). She will pursue research at Harvard University with Professor Richard H. Holm as an NIH postdoctoral fellow.



Stephen J. Lippard, a Massachusetts Institute of Technology Professor of Chemistry, completed a Bachelor of Arts magna cum laude at Haverford College and a Ph.D. in Chemistry at MIT. After a postdoctoral year at MIT, he served on the Chemistry faculty at Columbia University. He spent sabbaticals at the University of Göteborg in Sweden and at the MRC Laboratory of Molecular Biology in Cambridge, England. He is a recent recipient of the International Precious Metals Institute's Henry J. Albert Award for his work on the interactions of platinum metals with nucleic acids, the American Chemical Society Award in Inorganic Chemistry, and the Remsen Award of the Maryland Section of the ACS. He was elected a Fellow of the American Association for the Advancement of Science and of the American Academy of Arts and Sciences. His professional activities include local section and divisional service to the American Chemical Society. He has chaired several symposia at ACS national meetings, and he has been Editor of the well-known series, *Progress in Inorganic Chemistry*, from Volume 11 to the present. Dr. Lippard serves as Associate Editor of *Inorganic Chemistry* and on the editorial boards of several other technical journals.

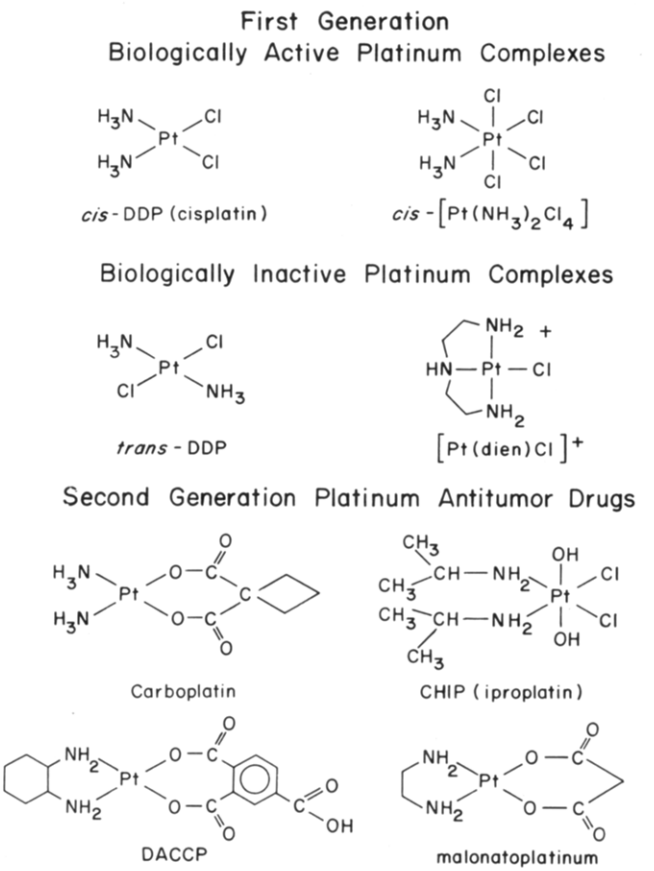


Figure 1. Structures and some trivial names and abbreviations for platinum complexes discussed.

across platinum electrodes immersed in an aerobic solution of *Escherichia coli* cells growing in the presence of NH_4Cl, the bacteria did not divide normally but grew into filaments up to 300 times their normal length.[15] An electrolysis product from the platinum electrode, identified as *cis*-$[Pt(NH_3)_2Cl_4]$,[16] was responsible for this intriguing behavior. Subsequently, various other platinum group metal complexes were found to induce filamentous growth in bacteria. Interestingly, while the divalent compound *cis*-$[Pt(NH_3)_2Cl_2]$ and related neutral *cis*-bis(amine)platinum(II) and -platinum(IV) complexes were active, the trans isomers were not. Instead, they suppressed bacterial growth at high concentrations.

Since the active platinum compounds suppressed cell division without killing the bacteria, it was conjectured that they might halt the rapid growth of tumor cells with little toxicity to the host animal. Testing of platinum compounds for antitumor activity in animals soon yielded parallel results; the cis isomers were active, while the trans isomers were not (Figure 1).[17,18] *cis*-DDP, in particular, was found to be active against a wide variety of animal tumors. Clinical trials ensued in 1972, culminating in FDA approval in 1979.

One of the most widely used anticancer drugs in the United States, Europe, and Japan today,[3] both by itself and in combination chemotherapy, *cis*-DDP has been successful in the treatment of bladder, lung, head and neck, cervical, and, especially, testicular and ovarian cancers.[4,5] Unfortunately, severe side effects accompany and often limit the clinical applications of this drug. Efforts to develop *cis*-DDP analogues having reduced toxicity and a broader range of therapeutic activity recently led to release of diammine(1,1-cyclobutanedicarboxylato)platinum(II), carboplatin, for use in the United Kingdom. Carboplatin displays activity similar to that of *cis*-DDP when used to treat ovarian cancer but is less toxic.[19] Other second-generation drugs, in-

cluding iproplatin (CHIP), DACCP, and malonatoplatinum, are currently undergoing clinical trials (Figure 1).[5] Meanwhile, intensive efforts have been directed toward elucidating the mechanism of anticancer activity of the parent and most widely used compound, cisplatin. Such studies may one day lead to the development of more successful anticancer drugs.

III. Mechanism of Action Studies of cis-$[Pt(NH_3)_2Cl_2]$

A. Structural Considerations

Both stereoisomers of diamminedichloroplatinum(II) (Figure 1) have two labile chloride ligands and two ammine ligands that are inert to substitution under biological conditions.[20] To the structural chemist, it is most intriguing that only the cis isomer displays anticancer activity. Moreover, most biologically active platinum complexes, whether square-planar or octahedral, contain two moderately labile cis leaving groups and two cis nitrogen donor amine ligands. Compounds with very labile leaving groups, such as nitrate ion, are more toxic and demonstrate considerably less activity. The trans isomers are always inactive. Monofunctional complexes, like chlorodiethylenetriamineplatinum(II), $[Pt(dien)Cl]^+$ (Figure 1), having just one labile ligand, are also inactive.

The implication of these findings is that the specific chemical reaction or reactions responsible for antitumor activity require bifunctional attachment to biological molecules. Thus, the identification of chemical interactions unique to the cis isomer in biological systems should provide important clues to unraveling details of the molecular mechanism of action of the drug.

B. DNA as the Cellular Target of *cis*-DDP

Numerous cellular components contain potential binding sites for heavy metals.[21] Among the first challenges researchers faced in studying *cis*-DDP were to determine its major targets and to identify which was most important for antitumor activity.

The induction of filamentous growth in bacteria by *cis*-$[Pt(NH_3)_2Cl_2]$ means that cell division is impaired without simultaneous inhibition of growth.[15] Other agents that produce similar effects, such as UV radiation[22] or hydroxyurea,[23] also an antitumor drug, were known to inhibit DNA synthesis.[23-25] Thus, a similar mechanism was suggested for platinum antitumor compounds. This theory was corroborated by experiments measuring the rates of synthesis of DNA, RNA, and proteins in cells treated with *cis*-DDP by monitoring the incorporation of radiolabeled precursors. At therapeutic doses of *cis*-DDP, DNA synthesis was preferentially inhibited over RNA and protein synthesis in both human AV_3 cells in culture[26] and in Ehrlich ascites cells in vivo.[27] The inactive trans isomer inhibited DNA synthesis to only a minor extent at similar doses.[26] Synthesis of DNA, RNA, and proteins by Ehrlich ascites tumor cells was measured in vitro at various intervals up to 96 h after injection of *cis*-DDP into mice. At 12–24 h following injection, the incorporation of radiolabeled uridine and L-leucine into RNA and proteins, respectively, began to recover. Restoration to normal synthesis of these components was virtually complete after 72–96 h. In contrast, the rate of incorporation of radiolabeled thymidine into DNA was persistently suppressed over the time period studied. These results suggested that selective and protracted inactivation of DNA synthesis is responsible for the drug's antitumor activity. Specifically, *cis*-DDP is thought to bind directly to DNA, rendering it unsuitable as a template for replication. Indirect inactivation of DNA synthesis caused by drug binding to DNA polymerase is not responsible for the observed effect, since preincubation of the enzyme with *cis*-DDP does not change its activity.[28]

More evidence supporting DNA as the cellular target of platinum drugs came from studies of lysogenic strains of *E. coli* bacteria. Under normal conditions, such bacteria repress genetic information previously encoded by viral infection. Various physical and chemical agents can derepress the viral genome, causing an active viral infection leading to cell lysis. Addition of low doses of *cis*-DDP to the bacterial growth medium resulted in lysis.[29] Use of *trans*-DDP did not. In another experiment, nonlysogenic bacterial cells previously treated with hydrolyzed *cis*-DDP were conjugated with lysogenic cells.[30] Conjugation resulted in the indirect induction of prophage and consequent cell lysis. Since DNA is the sole component entering the recipient cell following conjugation, platinum bound to this DNA had to be the agent that caused prophage induction. Studies on various other platinum compounds revealed a correlation between their antitumor activities and their abilities to induce prophage.[29] These results suggested that similar chemical reactions are responsible for the two phenomena, prophage induction and antitumor activity, and that both result from platinum–DNA interactions.

In another experiment, the survival of HeLa cells was measured as a function of the amount of *cis*-DDP bound to DNA, RNA, and proteins.[31] For both *cis*-DDP and *trans*-DDP, more platinum was bound to RNA than to DNA when expressed as moles of platinum per gram of biomolecule. When the molecular weights of each macromolecule were taken into account, however, it became apparent that much more platinum was bound to DNA than to RNA or protein on a per molecule basis. At pharmacologically relevant doses of *cis*-DDP, the levels of binding to RNA and protein were too low to inactivate all such molecules. For example, at a drug concentration where one inactivating event occurs, on the average, in each cell, only one out of every 1500 protein molecules will contain a single bound platinum atom, assuming no binding selectivity.[31] On the other hand, the observed levels of platinum bound to each molecule of DNA were sufficient to account for cell death, since DNA replication is a processive phenomenon.

Evidence indicates that *trans*-DDP, like cisplatin, enters cells and binds to DNA.[31-36] In cell culture and in vivo, however, *trans*-DDP inhibits DNA synthesis only at much higher doses than *cis*-DDP. There are several possible explanations for this phenomenon, including differential cellular uptake rates for the two isomers and/or repair of their DNA adducts. Another possibility is that adducts formed on DNA by *cis*-DDP are inherently more effective at inhibiting replication than those formed by *trans*-DDP. At present, there is conflicting information concerning which of these ex-

planations is responsible for the different biological activities of the two isomers. Perhaps more than one of these possibilities are important.

Mechanistic studies of the inhibition of viral DNA replication in SV40-infected African green monkey cells following treatment with either *cis*- or *trans*-DDP support the idea that cellular repair processes might discriminate between different adducts formed by the two isomers.[35] Inhibition of replication was measured by incorporation of radiolabeled thymidine, and the total amount of platinum associated with newly synthesized SV40 DNA at a given platinum concentration in the medium was measured by atomic absorption spectroscopy (AAS). The results of this study are displayed in Figure 2. As expected, 14 times more *trans*- than *cis*-DDP in the medium was necessary to produce the same inhibition of DNA replication (Figure 2A). Interestingly, however, equivalent levels of bound *cis*- and *trans*-DDP per nucleotide inhibited DNA replication to the same extent (Figure 2C). The differential effects on SV40 DNA replication caused by similar amounts of the two isomers in the cell medium can be readily understood by examining the binding curves in Figure 2B. Much higher levels of *trans*-DDP than *cis*-DDP in the medium are necessary to produce equivalent bound drug to nucleotide (D/N) ratios. Similar results were observed in studies of mouse L1210 cells,[34] where both *cis*- and *trans*-DDP inhibited cellular DNA replication by 50% at a D/N ratio of 2×10^{-4}.

To gain a better understanding of this phenomenon, the amount of platinum bound to cellular DNA as a function of time was measured in confluent CV-1 cells treated with 10 μM *cis*- or *trans*-DDP.[35] The cellular uptake of both isomers as a function of added platinum was also measured. The results of these experiments are shown in Figure 3. The amounts of *cis*- and *trans*-DDP found in CV-1 cells after 48 h of incubation with either 1, 5, or 10 μM platinum are equivalent (Figure 3A). Therefore, preferential cellular uptake is not responsible for the higher levels of *cis*- vs. *trans*-DDP bound to DNA. On the other hand, the kinetic results depicted in Figure 3B reveal striking differences between the two isomers. During the first 6 h of incubation, *trans*-DDP binds more rapidly to DNA than *cis*-DDP. In the next 6 h, however, the amount of bound *trans*-DDP rapidly diminishes and then continues to decrease at a slower rate for the remaining 36 h. The amount of bound *cis*-DDP continues to rise steadily for the entire measured time period.

These results suggest that *trans*-DDP adducts are preferentially repaired from DNA. Although *cis*-DDP and *trans*-DDP are both capable of forming adducts that interfere with normal DNA replication, those adducts formed by *trans*-DDP are more efficiently removed in the cell. Consequently, much higher doses of *trans*-DDP are required to produce an equivalent effect on DNA replication. The details of how *trans*-DDP adducts are removed are presently unknown.

A previous set of experiments investigating platinum binding to Chinese hamster ovary cells revealed kinetic profiles for *cis*- and *trans*-DDP binding to DNA very similar to those of Figure 3B.[32] At 5.5 μM *trans*-DDP in the culture medium, the amount of platinum bound per DNA nucleotide increased rapidly over the first 5–6 h of treatment and then dropped to less than one-fourth

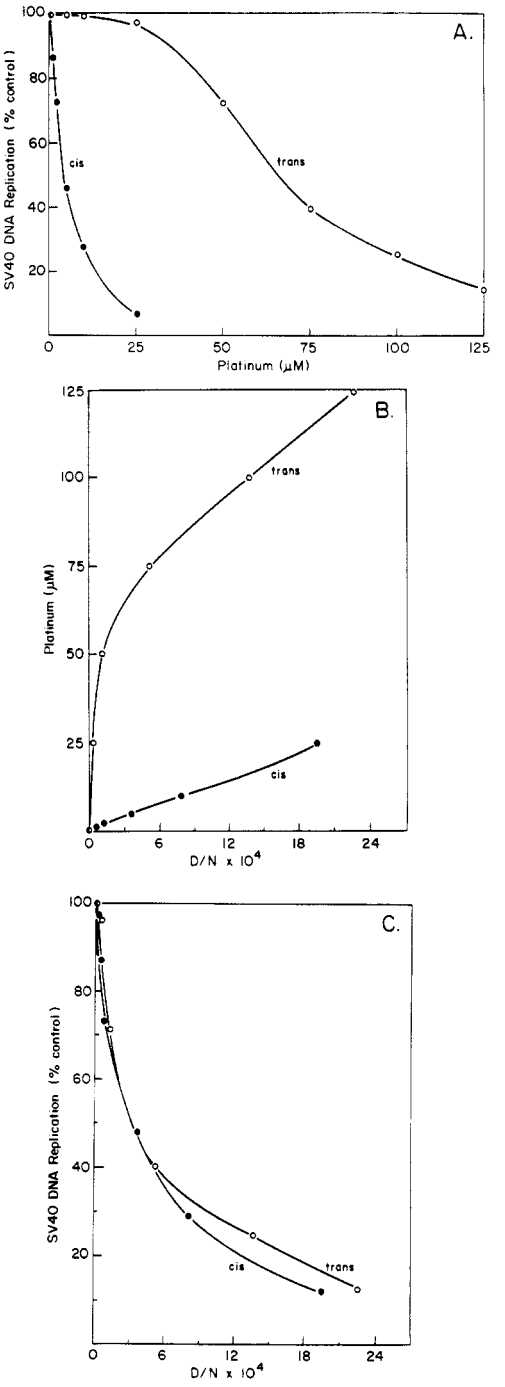


Figure 2. SV40 DNA replication in CV-1 cells as a function of platinum concentration in the medium (panel A) or D/N (panel C). In panel B, D/N is plotted as a function of platinum concentration in the medium. SV40-infected cells were treated with *cis*-DDP (●) or *trans*-DDP (○) at the indicated concentrations for 40 h. SV40 DNA replication relative to control (untreated) cells was measured by incorporation of [^{3}H]thymidine, added after the first 24 h of platinum treatment, and Pt in isolated SV40 chromosomes was measured by AAS. The data shown are from a representative experiment. Experiments were carried out in quadruplicate. Reproduced with permission from ref 35. Copyright 1985 American Chemical Society.

its maximum value during the next 10 h. The authors suggested that cell growth and concomitant DNA synthesis might account for this abrupt drop,[32] but their data showed the number of cells did not even double between the 5- and 16-h time points. Thus, DNA synthesis could only partially account for the abrupt reduction in bound *trans*-$\{Pt(NH_3)_2\}^{2+}$ per nucleotide. Repair of DNA adducts formed by *trans*-DDP may

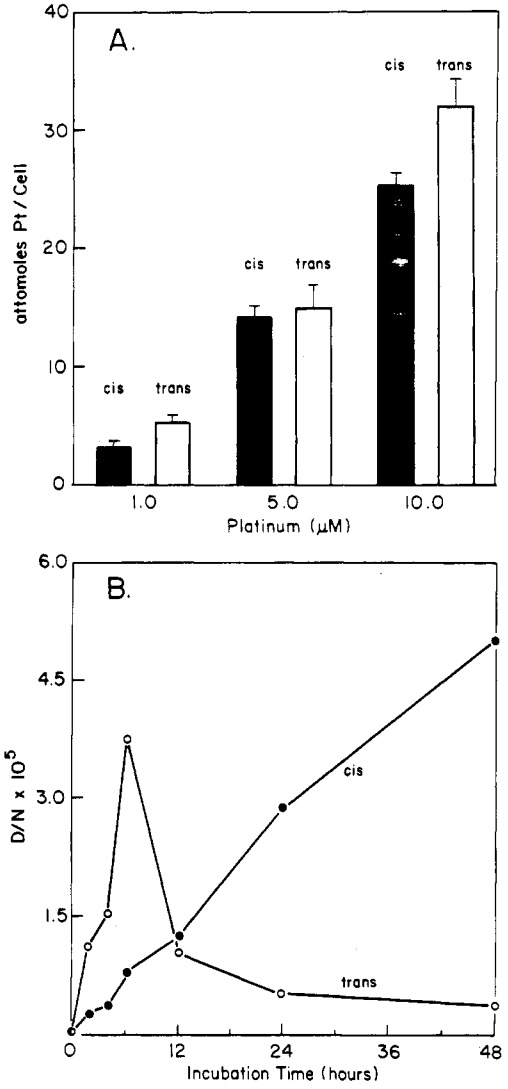


Figure 3. Uptake of platinum by CV-1 cells (A) and time course of platinum binding (B) to DNA of uninfected CV-1 cells. Uptake of *cis*-DDP (closed bars) and *trans*-DDP (open bars) at the indicated concentrations 48 h following addition of the compounds was measured by AAS of cellular acid digests. Over the time course of 0–48 h, cells were treated with 10 μM *cis*-DDP (●) or *trans*-DDP (○) and cellular DNA was purified and analyzed by platinum AAS. Error bars in (A) indicate the mean values of triplicate analyses. The time course measurements were performed in quadruplicate, and data from one representative experiment are shown in (B). Reproduced with permission from ref 35. Copyright 1985 American Chemical Society.

contribute to the observed behavior.

In a more recent report,[36] both *cis*- and *trans*-DDP were observed to bind DNA in CV-1 cells continuously with time over 48 h. There was no sudden drop in the level of bound *trans*-DDP that could be ascribed to differential repair. One possible explanation for the different results obtained by the various studies may be that, in the more recent work, *cis*- and *trans*-DDP were dissolved in dimethyl sulfoxide (Me_2SO) before their addition to the cell culture media.[36] As discussed in section IV.B.3, Me_2SO reacts rapidly with *trans*-DDP and alters its DNA binding properties. The authors of ref 36 seemed to be aware of the potential problems of using Me_2SO and attempted to control for them by carrying out an experiment in which *trans*-DDP was dissolved in phosphate-buffered saline. In this experiment, however, the protocol was different from that of Figure 3B, precluding any clear conclusions.

Differential repair could account for other characteristics of *cis*-DDP biochemistry, including its selectivity for tumor vs. normal cells, the various responses it elicits in different types of tumors, and the different responses of patients with similar cancers to cisplatin chemotherapy. Several *E. coli* strains[37–43] and a human cell line[44] lacking various pathways of DNA repair exhibit lower survival rates than normal cells when treated with *cis*-DDP. The pathways by which *trans*-DDP–DNA adducts are repaired from DNA in cells do not seem to be the same as those involved in repair of DNA platinated by *cis*-DDP.[43] Repair-deficient recA and uvrB *E. coli* mutants have a lower survival rate when treated with *trans*-DDP than repair-proficient cells, although not nearly as low a survival rate as the same cells treated with an equal amount of *cis*-DDP.[40] In other reports, the toxic effects of *trans*-DDP were similar in both DNA repair deficient and wild-type strains.[41,42] The results imply that *cis*-DDP interacts directly with DNA and that the resulting damage can be repaired by defined mechanisms. If so, the effects of *cis*-DDP treatment on tumor cells that may be deficient in their ability to repair DNA damage could account for its anticancer activity. Much more work is needed in this area.

Whatever the mechanisms by which *cis*-DDP is an active antitumor drug while *trans*-DDP is not, structural differences between Pt–DNA adducts formed by the two isomers appear to be involved, and the identification and characterization of such adducts should provide valuable information. Before discussing this subject, however, it is necessary first to describe details of the aqueous chemistry of platinum–ammine complexes.

C. Aqueous Solution Chemistry of *cis*-$[Pt(NH_3)_2Cl_2]$

In the earliest biological experiments, an initial insensitivity to *cis*-DDP, lasting about 2 h, was noted.[26,27] It was thus suggested that *cis*-DDP may not itself be the active species, but rather is converted over a period of time to the actual drug. Examination of the aqueous chemistry of *cis*-$[Pt(NH_3)_2Cl_2]$ provides an explanation for this behavior.

In water, the labile chloride ions are displaced by solvent molecules in a stepwise manner as shown in eq 1 and 2, with rate constants k_1 and k_2 approximately

$$cis\text{-}[Pt(NH_3)_2Cl_2] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} cis\text{-}[Pt(NH_3)_2Cl(H_2O)]^+ + Cl^- \quad (1)$$

$$cis\text{-}[Pt(NH_3)_2Cl(H_2O)]^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} cis\text{-}[Pt(NH_3)_2(H_2O)_2]^{2+} + Cl^- \quad (2)$$

2×10^{-5} and 3×10^{-5} s^{-1}, respectively.[45,46] Coordination of water to platinum lowers its pK_a, causing hydroxo products to form, as well.[47,48] Multinuclear hydroxide-bridged species also form[49,50] but are unlikely to be a major intracellular component at biological platinum concentrations.

From the equilibrium constants and acid dissociation constants for the various products formed by [Pt(en)Cl_2] under physiological conditions, the relative amounts of these products in plasma and cells were estimated.[48] In plasma, where the chloride ion con-

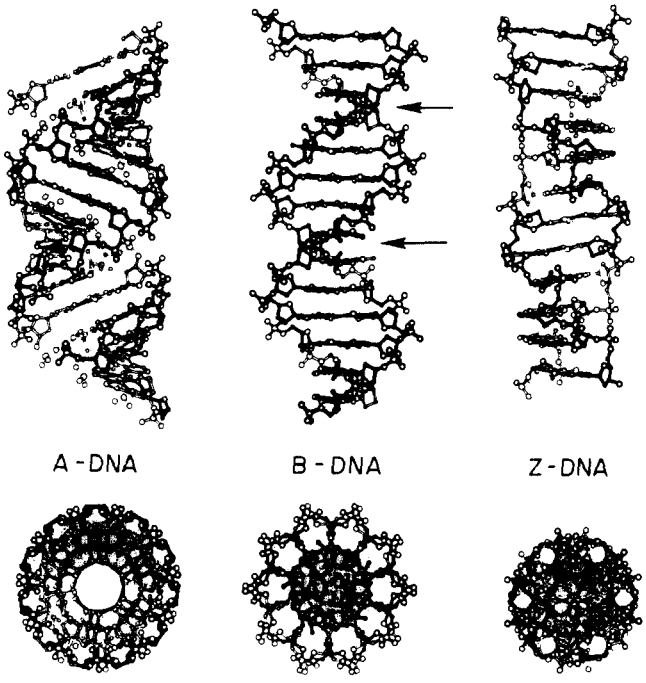


Figure 4. Ball and stick representations of side and top views of A-DNA, B-DNA, and Z-DNA. For B-DNA, arrows near the top and bottom of the helix point to the minor and major grooves, respectively. Reproduced with permission from ref 8. Copyright 1984 Springer-Verlag.

centration is high (103 mM), the predominant species is the neutral dichloro complex, which can passively diffuse across cell membranes. Inside the cell, the chloride ion concentration drops to about 4 mM, allowing the various hydrolysis products to form. At least one aqua group is present in 42% of the platinum complexes at 4 mM chloride ion concentration. Since water is a much better leaving group than chloride[51,52] and hydroxo groups are inert to substitution in platinum complexes,[52] aqua species are those most likely to react with DNA. These positively charged platinum complexes may be electrostatically attracted to the negatively charged DNA helix. Thus, the limiting step for reaction of *cis*-DDP with DNA is hydrolysis, which controls the time dependence of platinum binding to DNA.[53,54] For example, at an added formal ratio of 0.055 platinum atom/nucleotide at pH 7.4 and 37 °C in 1 mM phosphate and 3 mM NaCl, 0.017 platinum atom is bound/nucleotide after 3 h. Under these conditions, platinum binding levels off after 24 h, at 0.043 bound platinum atom/nucleotide.[54] The rate of dissociation of platinum from DNA is extremely slow at 37 °C. Thus, binding of *cis*-DDP to DNA is kinetically, rather than thermodynamically, controlled. *trans*-DDP exhibits similar kinetics of binding to DNA as *cis*-DDP in vitro.[53]

IV. Interactions of Platinum Compounds with DNA

A. DNA Structure and Platinum Binding Sites

The surface of the DNA double helix is characterized by major and minor grooves (Figure 4). The backbone of each DNA strand is composed of repeating deoxy-

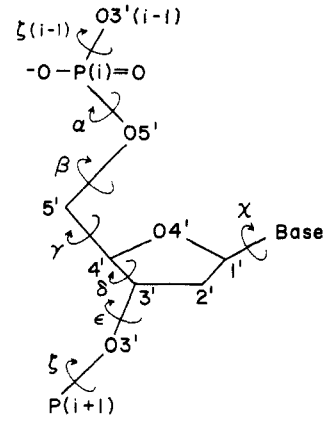


Figure 5. Schematic of the DNA backbone, showing deoxyribose ring-numbering schemes and torsion angles α–ζ and χ.

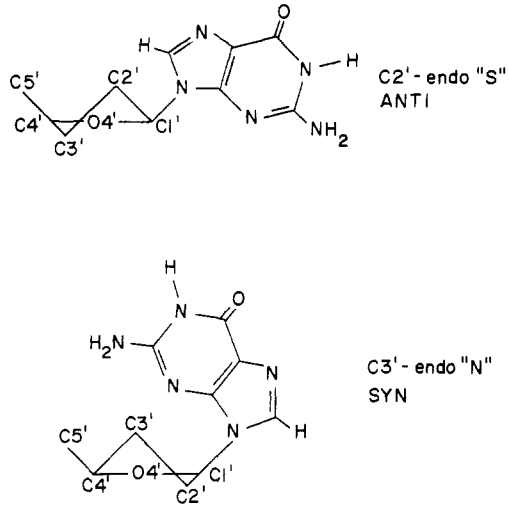


Figure 6. Schematic of the two main classes of sugar pucker and the two classes of base orientations commonly found in nucleic acid structures.

ribose phosphodiester units. In Figure 5 is displayed a schematic of the backbone containing the numbering of the deoxyribose rings, the backbone conformational angles α–ζ, and χ, the torsion angle about the glycosyl bond. The DNA backbone has polarity; in Figure 5, the 5′-end of the molecule is at the top and the 3′-end is at the bottom.

A continuum of possible sugar ring puckers exists, described by the pseudorotation cycle.[8,9] The pseudorotation angle, P, defines which of these occurs in a given molecule. The amplitude, ψ, defines the degree of pucker. In general, two classes of sugar puckers are found (Figure 6). In hydrated crystalline fibers and in solution (B-DNA), the C2′-endo pucker predominates, where the 2′- and 5′-carbon atoms are on the same side of the plane defined by C1′, C4′, and O4′. In higher salt fibers (A-DNA) and in RNA, the C3′-endo pucker occurs, where C3′ and C5′ are on the same side of the plane. The former pucker is often referred to as the "N" conformation, and the latter as the "S" conformation, corresponding to their respective positions at the "north" and "south" ends of the pseudorotation cycle.

The N9 atoms of the purine bases, adenine and guanine (Figure 7), are attached via the glycosyl bond to the 1′-carbon atoms of the DNA backbone. The pyrimidine bases, cytosine and thymine, bond to C1′ through their N1 atoms. Rotation about the glycosyl

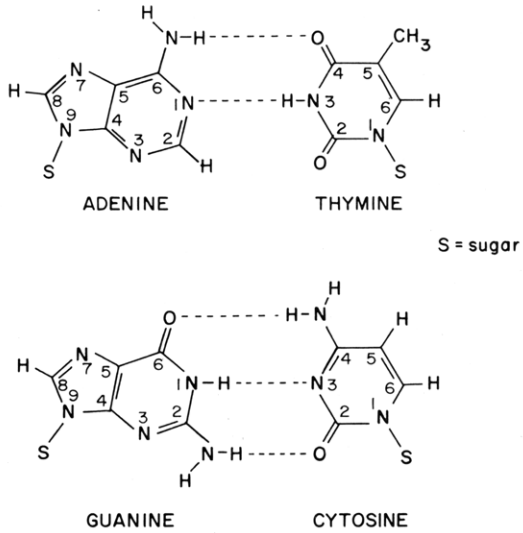


Figure 7. Schematic of Watson–Crick G-C and A-T base pairs, showing the atom-numbering schemes. In these views down the helix axes, the major grooves of DNA are pointing toward the top of the figure and the minor grooves are pointing toward the bottom of the figure.

bond by torsion angle χ results in one of two major conformations. In the syn conformation, the bulky part of the base is oriented toward the sugar, whereas in the anti conformation the bulky part of the base is oriented away from the sugar (Figure 6). Under normal conditions, the anti conformation is of lower energy. Each unit consisting of a phosphate, a deoxyribose, and a base is called a mononucleotide. In an oligonucleotide of defined length, the nucleotide at the 5′-end is referred to as N(1), where N = G, A, T, or C, the adjacent nucleotide is referred to as N(2), and so on.

The double helix is stabilized by hydrogen bonds between guanine and cytosine and between thymine and adenine of opposite strands and by stacking interactions between parallel bases perpendicular to the helix axis. Figure 7 depicts the most common hydrogen-bonding interactions between paired bases and their numbering schemes. Looking down the helix axes in Figure 7, the major groove of DNA is at the top of each base pair and the minor groove is at the bottom.

There are a variety of binding sites available to heavy metals on DNA.[55] Metals may covalently bind to the negatively charged phosphate oxygen atoms and to the nitrogen and oxygen atoms of the purine and pyrimidine bases. Planar molecules can intercalate between DNA base pairs.[56]

Early experiments revealed a shift of the absorbance maximum of DNA from 259 to 264 nm upon reaction with platinum compounds, indicating platinum association with the bases.[57] Moreover, *cis*-DDP noncompetitively inhibits the binding of the intercalator, ethidium bromide, to DNA. The platinum complex therefore binds covalently and not by intercalation.[58,59] Although platinum can bind to the phosphates of mononucleotides,[60–62] the pyridine-like and imidazole-like nitrogen atoms have a greater affinity for the polarizable Pt(II) atom,[63,64] and it is therefore less likely that phosphate coordination will occur in a competitive binding situation.

X-ray diffraction and NMR studies of complexes formed between platinum and N9-alkylated purines, N1-alkylated pyrimidines, nucleosides, and nucleotides

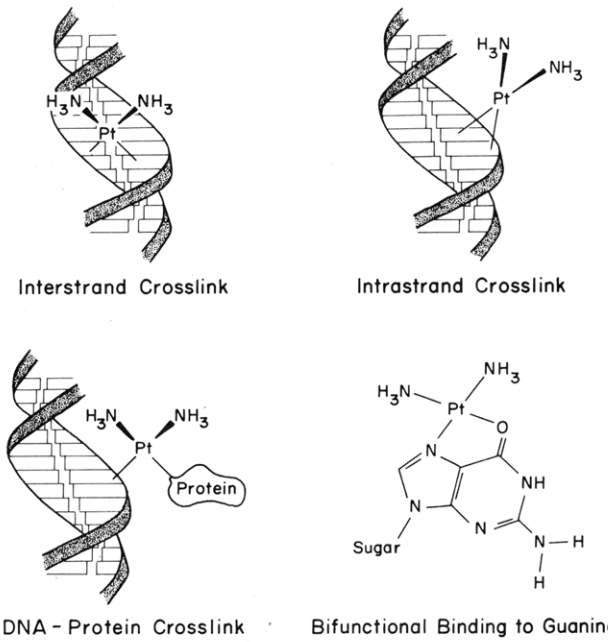


Figure 8. Possible bifunctional binding modes of *cis*-DDP with DNA.

have been used to elucidate platinum binding sites.[12,65] Under neutral conditions, platinum binds to the N7 atom of guanine, the N7 and N1 atoms of adenine, and the N3 atom of cytosine. In DNA, atoms involved in base pairing, i.e., N1 of adenine and N3 of cytosine, are less available for metal binding than the more exposed sites in the grooves. By Raman difference spectrophotometry, the order of reactivity in competitive reactions of the four nucleoside monophosphates with *cis*- and *trans*-DDP was established as GMP > AMP ≫ CMP > UMP, confirming that N7 of GMP is the preferred binding site.[66] Electrostatic potential calculations indicate that the guanine carbonyl group enhances the basicity of N7, while the amino group in adenine reduces the relative basicity of N7.[67,68] Recent quantum mechanical calculations of bond energies for platinum–base binding support these conclusions.[69] In DNA, N7 of guanine is exposed on the surface of the major groove (Figure 7), making it very accessible to metal binding.

B. Binding Modes of *cis*- and *trans*-DDP with DNA

Various possible bifunctional binding modes of *cis*-DDP with DNA are represented in Figure 8. Since *cis*- and *trans*-DDP are expected to form similar monofunctional adducts, and because platinum complexes such as $[Pt(dien)Cl]^+$ are inactive as antitumor drugs and ineffective at blocking DNA replication in vitro,[70] monofunctional adducts are unlikely to be responsible for the biological activity of *cis*-$[Pt(NH_3)_2Cl_2]$. Instead, bifunctional binding to two sites on a single base, cross-links between two bases on opposite strands of the DNA helix, DNA–protein cross-links, and intrastrand cross-links between two bases on the same DNA strand have all been proposed as being responsible for the antitumor activity of *cis*-DDP.

1. Bifunctional Binding to a Single Base

Some of the earliest proposed binding modes involved platinum coordination to two sites on a single base.[71]

Among these, the most popular was an N7–O6 chelate on guanine, which would alter the normal Watson–Crick hydrogen bonding of the G-C base pair in duplex DNA.[72-78] To date, there is no X-ray crystallographic or NMR support for such a structure,[79,80] and the N7–O6 chelate has generally been rejected on geometric grounds.[55] Recently, however, X-ray structures were reported of two Pt(IV) complexes in which the metal was chelated to deprotonated 1-methylcytosine (1-MeC$^-$) through its N3 and N4 atoms.[81] The four-membered chelate rings in these structures resulted in unusual bond angles at platinum (64–116°) and in the 1-MeC$^-$ internal ring angles. Thus, steric arguments against formation of an N7–O6 chelate should be used with caution. Nevertheless, it is unlikely that such a binding mode would compete with other, more favorable binding interactions under normal conditions. Theoretical calculations support this conclusion.[69]

2. Interstrand Cross-Linking

Interstrand cross-linking of DNA in vivo and in vitro[31,82-90] has been measured by both denaturing gradient centrifugation and alkaline elution techniques. By analogy to bifunctional DNA alkylating agents,[91] which display many of the same biological properties as *cis*-DDP, such cross-links have been postulated to be responsible for the activity of platinum anticancer drugs. While a correlation exists between interstrand cross-linking by *cis*-DDP and cytotoxicity in some experiments,[84-87] it does not in others.[87,88] Moreover, in none of these studies was intrastrand cross-linking measured, the binding mode most prevalent among all adducts formed by *cis*-DDP (vide infra). Recent estimates indicate that DNA interstrand cross-links formed in mammalian cells constitute less than 1% of total Pt–DNA adducts shortly after treatment.[89] Statistically, this percentage is too low to account for the cytotoxicity of *cis*-DDP.[92,93] The survival of cells transformed with pBR322 DNA modified by an average of 5.6 atoms of bound platinum/genome is reduced to only 1% of survival of untreated plasmid DNA.[43]

In recent work, interstrand cross-links formed by *cis*-DDP were proposed to be important cytotoxic events, owing to their persistence in certain cell lines.[89,94] The fact that *trans*-DDP also forms interstrand cross-links, although apparently more easily repaired than those formed by *cis*-DDP,[84,85,89] makes it unlikely that such adducts are responsible for the activity of cisplatin. Moreover, any relationship drawn between cytotoxicity and interstrand cross-linking must also take into account the more prevalent intrastrand cross-links.

From experiments carried out to identify the adducts produced by binding of *cis*-DDP to DNA (vide infra), it has been demonstrated that interstrand cross-linking occurs predominantly between two guanine N7 atoms on opposite strands.[95] Further information about the chemical and structural nature of these cross-links is desirable.

3. DNA–Protein Cross-Linking

Cross-links between DNA and proteins also comprise only a small fraction (0.15%) of the total Pt–DNA adducts formed in mammalian cells.[89] Both *cis*- and *trans*-DDP cross-link nuclear DNA and non-histone chromosomal proteins.[35,96] *trans*-DDP forms more such cross-links than *cis*-DDP at equivalent D/N ratios. There is no correlation between inhibition of DNA synthesis and DNA–protein cross-linking[35,84,85,97] which, along with their low frequency, indicates that DNA–protein cross-links are unlikely to be responsible for the antitumor activity of *cis*-DDP. At this time, nothing is known about the identities of donor atoms on proteins involved in DNA–protein cross-linking. Likely possibilities are nitrogen atoms of histidine and the sulfur atoms of cysteine and methionine side chains, since platinum has an affinity for sulfur donors.[98]

The propensity of platinum for making links to sulfur reflects a problem associated with many biological experiments with *cis*- and *trans*-DDP, including some of those designed to measure interstrand and DNA–protein cross-linking. In such experiments, dimethyl sulfoxide (Me_2SO) was used to dissolve the platinum compounds.[31,36,82,89,99-101] It has recently been demonstrated that solvolysis reactions of *cis*- and *trans*-DDP in Me_2SO at 37 °C proceed with half-lives of 60 and 8 min, respectively, to yield *cis*-$[Pt(NH_3)_2(Me_2SO)Cl]^+$ and *trans*-$[Pt(NH_3)_2(Me_2SO)Cl]^+$.[102] When the reactions of *trans*-DDP with calf thymus DNA and with the synthetic oligonucleotide d(GpTpG) were studied following dissolution of the platinum complexes in Me_2SO or aqueous buffer for only 5 min, significant differences were observed both in the rate of platinum binding and in the assortment of products obtained.[102] Thus, some interpretations of results of experiments designed to study the mechanism of action of platinum antitumor drugs in which Me_2SO is employed may be erroneous.

4. Intrastrand Cross-Linking

Intrastrand binding of *cis*-DDP to the N7 atoms of two adjacent guanosine nucleosides occurs more frequently in vitro and in vivo than any other binding mode. The intrastrand d(GpG) cross-link was correctly forecast by early buoyant density measurements of a mixture of double-stranded, naturally occurring DNAs containing different (G + C) contents. Buoyant densities increased in proportion to both the amount of added platinum and to the (G + C) content of the DNA.[103] The notion that *cis*-DDP preferentially binds to guanosine was supported by experiments using both spectrophotometry[104] and ^{195}Pt-labeled *cis*-DDP[105] to monitor its reaction with DNA. Extension of the buoyant density experiments to the homocopolymer, poly(dG)·poly(dC), and the alternating heterocopolymer poly(dG·dC) containing bound *cis*-DDP, revealed the buoyant density of modified poly(dG)·poly(dC) to be much greater than that of the platinated alternating heterocopolymer.[106] This interesting result was attributed to preferential binding of platinum to oligo(dG) vs. mono(dG) sites on the DNA. *trans*-DDP also increases the buoyant densities of DNAs in proportion to their (G + C) contents but, unlike *cis*-DDP, does not preferentially increase the buoyant density of poly(dG)·poly(dC) over poly(dG·dC). It was concluded that both isomers bind equally well to individual guanine bases but that only *cis*-DDP is selective for adjacent guanosines.[106] An alternative explanation is that *cis*-DDP binds to both individual guanosines and d(GpG) sequences equally well but produces structures at the latter sites that more severely affect DNA buoyant density. This argument can be extended to explain the

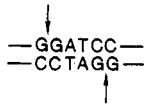
TABLE I. Distribution of Various Adducts Formed between *cis*-DDP or [^{3}H][Pt(en)Cl$_2$][a] and DNA in Vitro and in Vivo

D/N	total incubn time (temp, °C)	adducts formed, % *cis*-[PtA$_2${d(pGpG)}][b]	*cis*-[PtA$_2${d(pApG)}][b]	*cis*-[PtA$_2${d(GMP)$_2$}][b]	monofunctional adducts, %	remaining Pt,[c] %	ref
			In Vitro				
0.055	5 h (50)	47–50	23–28	8–10	2–3	10	121[d]
0.022	5 h (50)	60–65	20	~4	~2	9–14	121[d]
0.01	16 h (37)	62	21	7		10	123[e]
f	30 min (37)	36	3	8	40	13	123[e]
f	2 h (37)	54	9	9	14	14	123[e]
f	3 h (37)	57	15	9	4	15	123[e]
			In Vivo				
g	1 h (37)	35.9 ± 4.7[h]	<34[i]	3.1 ± 1.6[h]	38.5[i]	~22	141[d]
g	25 h (37)	46.6 ± 6.8[h]	<48[i]	3.0 ± 0.9[h]	<14.5[i]	~50	141[d]

[a] A radiolabeled analogue of *cis*-DDP, [[^{3}H]dichloroethylenediamine]platinum(II). [b] A$_2$ represents either (NH$_3$)$_2$ or ethylenediamine. [c] By difference. [d] Percentage of adducts based on total amount of platinum eluted from the separation column. [e] Percentage of adducts based on total amount of radioactivity eluted from the separation column. [f] Not given. [g] Chinese hamster ovary cells treated with 83 μM *cis*-DDP. [h] Results from ELISA. [i] Results from AAS. Where the AAS signal was too weak for reliable quantitation, the maximal amount possible is given.

increased buoyant densities of DNAs with higher (G + C) contents upon platinum binding.

In order to explore more thoroughly the regioselectivity of platinum binding to DNA, methods were developed in which sequence-specific DNA-cleaving enzymes were presented platinated DNAs as substrates. In the first such experiments the restriction endonuclease, Bam HI, which cleaves the sequence



was found to be inhibited by bound *cis*-{Pt(NH$_3$)$_2$}$^{2+}$.[107] Cutting by the endonuclease Pst I was also selectively inhibited at a site adjacent to an oligo(dG) sequence on the particular DNA plasmid used.[108] To study these effects more closely, platinated DNA labeled with ^{32}P at the 5′-end of one strand was digested by exonuclease III, an enzyme that progressively cuts DNA from its 3′-ends. Denaturation of the exonuclease III produced fragments and separation by size on a sequencing gel revealed that enzymatic digestion was inhibited at oligo(dG) sequences on the platinated labeled DNA strand.[109,110] It was proposed that binding of platinum to oligo(dG) sequences alters the DNA structure at such sequences so as to inhibit enzymatic digestion.

In a related experiment, binding of *cis*-DDP to (dG)$_n$ ($n \geq 2$) sequences of a primed phage M13mp8 viral DNA template was found to inhibit the synthesis of the second DNA strand by the large (Klenow) fragment of DNA polymerase I.[70] *trans*-DDP also blocked DNA synthesis, although at different and less regiospecific sequences than *cis*-DDP. A preference for binding of *trans*-DDP to d(GpNpG) sequences, where N is any intervening nucleotide, was noted. Binding of the biologically inactive, monofunctional complex [Pt(dien)Cl]$^+$ had no effect on DNA synthesis in this system. Thus, intrastrand cross-linking of DNA at specific sequences by both *cis*- and *trans*-DDP is sufficient to inhibit DNA replication in vitro.

Although none of the foregoing experiments proved that *cis*-DDP reacts exclusively at oligo(dG) sequences, they did demonstrate the importance of the d(GpG) intrastrand cross-link. Meanwhile, NMR techniques were being used to characterize adducts of *cis*-{Pt(NH$_3$)$_2$}$^{2+}$ with short fragments of chemically synthesized RNA and DNA. Reaction of *cis*-DDP with the dinucleoside monophosphates (GpG), (IpI), or d(GpG) or the deoxydinucleotide d(pGpG) resulted in bidentate complexes chelated through the guanosine or inosine N7 atoms.[111–113] In two experiments where the reacting deoxyoligonucleotide, d(ApGpGpCpCpT)[114] or d(TpGpGpCpCpA),[115] contained all four of the DNA bases, platinum bound exclusively at the N7 atoms of both guanosine residues. Although the sequences contained no isolated guanosines, the experiment clearly demonstrated the predilection of *cis*-DDP for binding to adjacent guanine over adenine, thymine, and cytosine bases.

Experiments in which platinated DNA is degraded either by enzymatic means or by acid hydrolysis have been invaluable in identifying and quantifying Pt–DNA adducts.[95,116–123] The distribution of species observed in some of these studies is summarized in Table I. In one study, the adducts were unambiguously identified by NMR spectroscopy and by comparison with chemically synthesized compounds.[121] *cis*-DDP was allowed to react with salmon sperm DNA at an added D/N ratio of 0.055 for 5 h at 50 °C. After inactivation of any monofunctionally bound platinum with NH$_4$HCO$_3$,[124] the DNA was completely degraded by deoxyribonuclease I and nuclease P1 to mononucleotides and platinum-containing oligonucleotides, which were separated by anion-exchange chromatography. The main separated adduct was identified as *cis*-[Pt(NH$_3$)$_2${d(pGpG)}]. Other identified products were *cis*-[Pt(NH$_3$)$_2${d(pApG)}], *cis*-[Pt(NH$_3$)$_2${d(GMP)$_2$}], and *cis*-[Pt(NH$_3$)$_3${d(GMP)}]$^+$ (Table I). Since the reference compounds, *cis*-[Pt(NH$_3$)$_2${d(GpCpG)}], and *cis*-[Pt(NH$_3$)$_2${d(GpApG)}], in which platinum is bound to the N7 atoms of the two guanosines, are digested by the same enzymes to *cis*-[Pt(NH$_3$)$_2${d(Guo)}{d(GMP)}] and mononucleotides, the presence of *cis*-[Pt(NH$_3$)$_2${d(GMP)$_2$}] among the digestion products of platinated DNA could arise from both interstrand cross-links and intrastrand cross-links of two guanosines separated by a third nucleoside. Unidentified adducts constituted approximately 10% of the total platinum eluted from the anion-exchange column and were assigned as oligonucleotide complexes resistant to enzymatic degradation, owing to their high platinum contents.[121] At a lower D/N ratio of 0.022, the same products resulted, although in slightly different proportions (Table I).

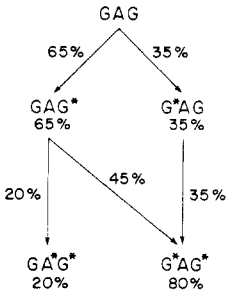


Figure 9. Two steps of reaction of *cis*-DDP with d(GpApG). The asterisks indicate platinum binding sites. The 65:35 distribution of 3′- and 5′-platinated adducts, respectively, in the first step of the reaction was postulated by analogy to results for $[Pt(dien)Cl]^+$ binding to d(GpApG).[129] Redrawn from ref 129.

A statistical analysis of the percentage of isolated relative to adjacent guanosines expected to occur in salmon sperm DNA illustrates the selective binding of *cis*-DDP to $(dG)_n$ ($n \geq 2$) sequences.[121] If all guanosines were equally reactive toward the platinum complex, then a much broader distribution of products, and a markedly reduced number of *cis*-$[Pt(NH_3)_2\{d(pGpG)\}]$ adducts, would have formed. The lower percentage of these adducts formed at the higher platinum binding level of 0.055 (Table I) indicates saturation of the oligo(dG) sequences, resulting in an increase of the relative proportion of the secondary product, *cis*-$[Pt(NH_3)_2\{d(pApG)\}]$.

This secondary adduct, in which platinum is bound to N7 of adenosine at the 5′-end and N7 of guanosine at the 3′-end of the dinucleotide, occurs in substantial proportions, even at low levels of added platinum (Table I).[120-123] In more extensive exonuclease III mapping experiments,[125] and analogous work using the 3′ → 5′ exonuclease activity of T4 DNA polymerase,[126] the d(ApG) adduct was also detected. Surprisingly, the d(GpA) intrastrand cross-link, having platinum coordinated to a 5′-guanosine and a 3′-adenosine, has never been observed in reactions of *cis*-DDP with DNA, although its formation has been reported with the dinucleoside monophosphate, (GpA).[127,128]

In a study of the interaction of *cis*-DDP with d(GpApG), two products were obtained and characterized.[129] In the first product, formed in 80% yield, platinum is bound bifunctionally to the N7 atoms of both guanosines in a 1,3-intrastrand cross-link (vide infra). The second product, a 1,2-intrastrand adduct in which platinum is bound to the N7 atoms of A(2) and G(3), was formed in 20% yield. Again, there was no evidence for a G(1)–A(2) complex. Neither the reaction temperature (30, 50, 80 °C) nor prior treatment of the platinum drug with $AgNO_3$ influenced the product ratio. A stepwise reaction occurs when *cis*-DDP interacts with (GpA) or (ApG).[128] From parallel studies of the reaction between $[Pt(dien)Cl]^+$ and d(GpApG), the first step in the reaction between *cis*-DDP and d(GpApG) was postulated to be platinum coordination to either the 5′-guanosine or 3′-guanosine (Figure 9).[129] The selective binding of $[Pt(dien)Cl]^+$ to the 3′- rather than the 5′-guanosine was explained by the "directing effect" of its 5′-phosphodiester link,[130] which is absent for the 5′-guanosine. Enhancement of binding at the 3′-guanosine would not be expected to occur in natural DNAs, where end effects are minimal. In the second step of the reaction,[129] platinum bound monofunctionally to the 5′-guanosine does not coordinate to the adjacent adenosine but only to the 3′-guanosine to form the 1,3-adduct. Platinum bound to the 3′-guanosine binds additionally either to the adjacent adenosine to produce the 1,2-adduct or to the 5′-guanosine to produce the 1,3-adduct.

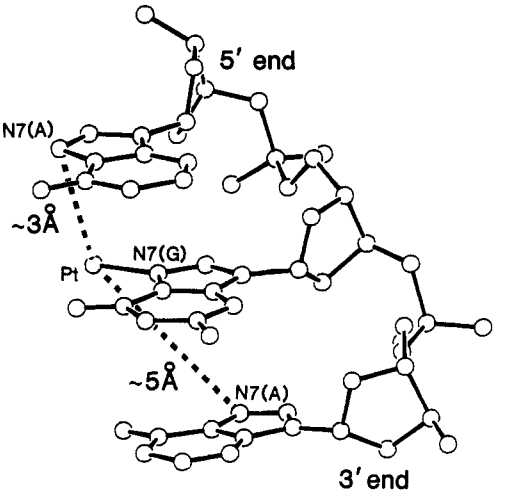


Figure 10. Diagram of a d(ApGpA) sequence in B-DNA, depicting a platinum atom bound to N7 of the G residue where Pt–N7 (G) = 2.0 Å. The platinum atom is ~3 Å from N7 of the A residue to its 5′-side and ~5 Å from N7 of the A residue to its 3′-side. Reproduced with permission from ref 131. Copyright 1984 American Chemical Society.

The following interesting explanation for the failure of a platinated 5′-guanosine to close to an adjacent 3′-adenosine has been proposed.[131] After initial reaction of *cis*-DDP with guanosine in DNA or RNA having, for example, an (ApGpA) sequence, the platinum atom is about 3 Å from N7 of the adenosine residue to its 5′-side and about 5 Å from N7 of the adenosine residue to its 3′-side (Figure 10). Therefore, reaction with the former site to produce the (ApG) adduct is preferred. The relatively long distance between a platinum atom bound to N7 of guanosine and the N7 atom of an adenosine residue to its 3′-side rationalizes the absence of platinated d(GpA) sequences in DNA following *cis*-DDP binding. This explanation does not take into account, however, any disruption of the local structure that might occur when platinum binds monofunctionally to guanosine.

In several studies,[121,123] it was observed that more P1 nuclease is required to digest regions of DNA containing the d(pApG)–platinum adduct than those containing other adducts. This result is of interest in terms of the relative lethalities of the various adducts. It is possible that enzymes in the cell responsible for DNA repair may have greater difficulty in removing platinated d(ApG), compared to other platinum–DNA adducts. The main structural difference between adenosine and guanosine coordinated to platinum is the exocyclic amino group at the 6-position of the former and the exocyclic keto oxygen at the 6-position of the latter. It has been postulated that the different steric properties of the exocyclic amino group discourage the formation of the d(ApG) relative to the d(GpG) adduct.[132]

Recent findings have suggested that the *cis*-$[Pt(NH_3)_2\{d(ApG)\}]$ adduct may be more mutagenic than the *cis*-$[Pt(NH_3)_2\{d(GpG)\}]$ adduct when formed within the tetracycline-resistant gene of pBR322 DNA.[133] The mutation analysis was simplified by platinating a 276-

TABLE II. Mutational Spectrum of *cis*-DDP-DNA Adducts[133]

proposed Pt adduct	total no. obsd mutations	local sequence (no. occurrences)[a]	mutation specificity
d(ApG)	14	AT*A*GGC (3)	A-T → T-A
		GA*A*GTG (2)	A-T → T-A
		AC*A*GTC (1)	A-T → T-A
		GG*A*GAT (1)	A-T → T-A
		GT*A*GGT (1)	A-T → G-C
		TT*A*GGA (2)	A-T → T-A, A-T → G-C
		GG*A*GTC (4)	A-T → T-A
d(GpG)	9	GAG*G*AT (1)	G-C → A-T
		CCG*G*CC (1)	G-C → C-G
		CCG*G*CA (1)	G-C → A-T
		TCG*G*GC (2)	G-C → A-T, G-C → T-A
		ACG*G*GCC (1)	G-C → T-A
		ACG*G*GCC (1)	G-C → T-A
		CCG*G*GGG (1)	G-C → T-A
		AAG*G*GAG (1)	G-C → A-T
d(GpCpG)	1	ATGC*G*AC (1)	G-C → T-A

[a] The mutated base is italicized.

base-pair restriction fragment located in the tetracycline resistance gene of the plasmid. The modified restriction fragment was ligated back into the plasmid, and the resulting material was used to transform SOS-induced (UV irradiated) or uninduced *E. coli* cells. A strong dose-dependent increase in tetracycline-sensitive mutants occurred only in the UV-irradiated host cells, indicating that SOS function is necessary for mutagenesis. Twenty-five mutants were sequenced and the positions and types of mutants catalogued. Twenty-four are single base-pair substitutions, and the remaining one is a −1 frameshift. Fourteen of the single base pair substitutions occur at d(ApG) sequences, nine occur at d(GpG) sequences, and one occurs at a d(GpCpG) sequence (Table II). If mutations occur solely at bases to which platinum is bound, then, according to this forward mutation assay, platinated d(ApG) is much more mutagenic than platinated d(GpG), since the latter adduct occurs more frequently than the former in DNA treated with *cis*-DDP (Table I).[133] If, on the other hand, mutations can occur at bases adjacent to, or between, platinated nucleosides, then the data obtained from this experiment are not so simply interpreted. For example, examination of the most highly mutated sequence, GG*A*G, where a total of five mutations occur, reveals that a d(GpG)–platinum adduct immediately adjacent to the mutated adenosine, or a 1,3-intrastrand cross-link of two guanosines separated by the mutated adenosine, could also explain the results. Further studies are necessary to clarify this point.

The 1,3-intrastrand cross-link, in which two coordinated guanosines are separated by a third nucleoside (A, C, T), was originally postulated to occur on the basis of mutation induction experiments of *cis*-DDP in the lac I system.[40] Of the mutations after treatment with *cis*-DDP 70% occurred at d(GpApG) or d(GpCpG) sequences. As recently pointed out, however, in the lac I assay, only positions on the genome of base substitutions leading to nonsense codons can be analyzed.[133] The failure to detect base substitutions at the 5′-end of d(ApG) sequences, suggested by the previously described assay to be highly mutagenic, may be due to the fact that only a single in-frame d(ApGpA) codon, the only sequence containing d(ApG) that could result in a nonsense codon, is contained in the lac I gene. Moreover, the position of the in-frame d(ApGpA) codon is too close to the C-terminal end of the coded protein to result in its inactivation. Thus, the relative mutagenicity of 1,3-d(GpApG) and d(GpCpG) adducts may have been overestimated.[40]

Substitution of aquated *cis*-DDP or *trans*-DDP by two GMP molecules occurs in two steps, with the second step being relatively slow.[79,130,134-136] If reaction of *cis*-DDP with DNA occurs by a similar mechanism, then monofunctional adducts would be expected to diminish with time, with largest amounts of such adducts occurring after relatively short reaction times. When 0.1 M NH_4HCO_3 was added during platination of DNA in order to quench the second step of the reaction, fairly constant relative amounts of the different adducts were observed during a 24-h time period.[121] Although the relative amount of monofunctional adducts varied slightly with time, bifunctional adducts predominated. The use of NH_4HCO_3 to inactivate monofunctionally bound platinum may be problematic, however. The inactivating reactions are carried out for 18 h at room temperature,[117] during which time it seems likely that most monofunctionally bound platinum atoms would close to form a second link to DNA. It is uncertain whether NH_3 could intercept this process fast enough to quench the second reaction step.

Other conditions have led to higher levels of monofunctional adducts.[123,137] Thiourea was used to trap monofunctional adducts of the radiolabeled analogue of *cis*-DDP, [^{3}H]*cis*-[Pt(en)Cl_2], with DNA.[123] After 15-min incubation of the platinum complex with DNA at 37 °C and a subsequent 10-min treatment with thiourea, 40% of the total radioactivity was found in a product containing thiourea following enzymatic degradation (Table I). After 2- and 3-h incubations of the platinum complex with DNA, the number of products containing thiourea dropped to 14% and 4%, respectively, of the bound platinum. The products containing thiourea were assigned to platinum bound monofunctionally to DNA.[123]

In a parallel kinetic study, radioactive guanosine was used to trap monofunctional adducts of *cis*- and *trans*-DDP with DNA.[137] After 2 h of reaction between various levels of the platinum complexes and DNA at 37 °C, approximately 15% of the adducts with the cis isomer contained radioactivity, in agreement with the experiments using thiourea, and about 80% of the adducts with the trans isomer contained radioactivity. These monofunctional platinum–DNA adducts disappeared surprisingly slowly with half-lives of 15 and 30 h, respectively. The preponderance of long-lived monofunctional adducts formed by *trans*-DDP observed in this experiment raises the interesting possibility that the vastly different biological activities of the two isomers result from formation of bifunctional adducts for *cis*-DDP, compared with monofunctional adducts for *trans*-DDP. If this hypothesis were true, the behavior of *trans*-DDP and of the known monofunctional compound [Pt(dien)Cl]$^+$ should be similar in biological experiments in which the platination reactions are carried out for short time periods. Further studies in which in vivo DNA-binding properties of both *trans*-DDP and [Pt(dien)Cl]$^+$ are compared to those of *cis*-

DDP are required to address this problem.

Until further information is available about the individual biological activities of the different adducts formed by *cis*-DDP with DNA, attention must be focused on the major adduct, the intrastrand d(GpG) cross-link. This and other 1,2-intrastrand adducts such as *cis*-[$Pt(NH_3)_2${d(ApG)}] are uniquely produced by *cis*-DDP and its analogues, since the labile sites of the trans isomer cannot link two adjacent purine N7 atoms. Evidence for the formation of the d(GpG) intrastrand cross-link in vivo is available from immunochemical studies. Antibodies have been elicited against calf thymus DNA treated with *cis*-DDP in vitro[138] and against the adduct *cis*-[$Pt(NH_3)_2$(Guo)(GMP)].[139] These antibodies have been used in a competitive enzyme-linked immunosorbent assay (ELISA)[140] to detect very small amounts of Pt–DNA adducts.

Antibodies raised against *cis*-[$Pt(NH_3)_2$(Guo)(GMP)] recognize both *cis*-[$Pt(NH_3)_2${d(GMP)}$_2$] and *cis*-[$Pt(NH_3)_2${d(pGpG)}], but not [$Pt(NH_3)_3${d(GMP)}]$^+$ or *cis*-[$Pt(NH_3)_2${d(pApG)}].[139] These antibodies were used to help quantitate and identify the digestion products of platinum-induced inter- and intrastrand cross-links in DNA obtained from *cis*-DDP-treated Chinese hamster ovary cells (Table I).[141] The isolated DNA was enzymatically degraded, and the products were separated by liquid chromatography. Although the levels of Pt–DNA adducts formed were too low for UV detection during the chromatographic analysis, adducts were identified in the fractions at which they were known to elute by competitive ELISA antibody assays. Platinum levels were also measured by AAS. When DNA was isolated immediately following a 1-h treatment with *cis*-DDP, ~38% of the total platinum per nucleotide was bound monofunctionally, as measured by AAS. Intrastrand d(GpG) cross-links accounted for ~36% of the bound platinum and the degradation product *cis*-[$Pt(NH_3)_2${d(GMP)}$_2$] resulting from interstrand crosslinking of two guanosines, and intrastrand cross-linking of two guanosines separated by one or more nucleosides accounted for ~3% of the total platinum, as measured by competitive ELISA. The quantity of d(ApG) intrastrand cross-links was too low to be measured by AAS, which is less sensitive than the competitive ELISA. After a posttreatment incubation of the cells for 24 h, the number of monofunctional adducts decreased below the level of detection by AAS, while the amounts of the other measured adducts remained almost constant.[141] This result was used to suggest that monofunctional adducts are easily repaired. On the other hand, monofunctional may be converting to bifunctional adducts that are subsequently repaired. On the basis of the total amount of DNA phosphate present in the eluate, the relative numbers of intrastrand d(GpG) cross-links decreased only very little, and after 24 h this adduct represented nearly half of the total amount of platinum bound to DNA (Table I).

Antiserum elicited against calf thymus DNA modified with *cis*-DDP in vitro detects Pt–DNA adducts in *cis*-DDP-treated cultured mouse leukemia L1210 cells, in L1210 cells from the ascites fluid of tumor-bearing mice exposed to *cis*-DDP, and in nucleated peripheral blood cells of cancer patients receiving *cis*-DDP chemotherapy.[140,142,143] The antibody does not recognize unmodified DNA, the drug alone, *trans*-DDP-modified DNA,

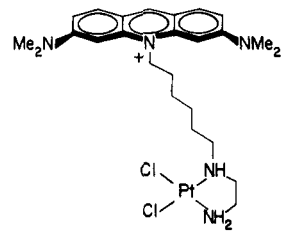


Figure 11. Schematic drawing of AO-Pt. Reproduced with permission from ref 149. Copyright 1984 American Chemical Society.

or monofunctional DNA adducts formed by [Pt(dien)Cl]$^+$. In fact, recognition appears to be specific for the d(GpG) cross-link, or other adducts such as the d(ApG) cross-link having similar stereochemistry. Studies with the homocopolymer poly(dG)·poly(dC) and the heterocopolymer poly(dG·dC) reveal immunoreactivity toward the former but not the latter.[144] Therefore, interstrand cross-links or intrastrand bifunctional adducts of two guanines separated by an intervening nucleotide that can be formed with both poly(dG)·poly(dC) and poly(dG·dC) are not recognized. Long-term studies of cancer patients suggested that those individuals who benefitted from *cis*-DDP chemotherapy were more likely to have measurable intrastrand cross-links than patients who failed chemotherapy,[142] further signifying the possible biological importance of this adduct.

C. Sequence Specificity of Platinum Binding

cis-DDP does not bind randomly to all oligo(dG) sequences in DNA. Rather, the electronic and/or molecular structures of the surrounding sequences must somehow be favorable, relative to other sequences, for binding by platinum. The dependence of DNA local structure on base sequence has been demonstrated in the X-ray crystal structure of a B-DNA dodecamer.[145] The remarkable structure of Z-DNA (Figure 4), favored by alternating purine and pyrimidine residues,[146] further illustrates this point.

In the previously described exonuclease III mapping experiments, an interesting phenomenon was observed. Although strong platinum-induced stops were observed at most oligo(dG) sites on a 165 base-pair restriction fragment of pBR322 DNA, at low D/N ratios only a relatively weak stop was observed at a 5′-d(G_6CG_2)-3′ sequence, 29 bases from the 3′-end of the molecule.[147] Pretreatment of the DNA with the intercalator, ethidium bromide, remarkably altered the exonuclease III sensitive binding of platinum to this sequence.[147] At low levels of ethidium, platinum binding occurred at the d(G_2) site in the sequence. With increased levels of ethidium, binding of platinum to the d(G_6) region was detected with a concomitant decrease in binding to the d(G_2) region. Additional ethidium did not increase the overall level of platinum binding but enhanced binding at the d(G_6CG_2) sequence. These effects are not the result of platinum complexation by ethidium bromide.[148]

Subsequently, the DNA binding regioselectivity of AO–Pt,[149] a complex in which [Pt(en)Cl_2] is covalently linked to the intercalator acridine orange by a hexamethylene chain, was studied.[125] The intercalating properties of this novel compound (Figure 11) induce

local modifications in DNA structure that influence the sequence specificity of platinum binding. The DNA-binding properties of AO–Pt, monitored by exonuclease III mapping, are similar to those of *cis*-DDP binding to DNA in the presence of ethidium, demonstrating that intercalator enhancement of platinum to the d-(G_6CG_2) site is caused by local modulations in the structure of the DNA. Enhanced binding to d(CGG) sequences of [Pt(en)Cl_2] in the presence of ethidium and of AO–Pt was also observed. These results illustrate that sequence-specific structure modulations in the vicinity of oligo(dG) residues can alter their accessibility to platinum binding. Addition of intercalators to modify these regional structures can render accessible for platinum a previously restricted site.

Interestingly, unlike platinum-linked acridine orange, free acridine orange does not enhance binding of *cis*-DDP or [Pt(en)Cl_2] to previously restricted sequences on DNA. In fact, of a series of phenanthridinium compounds and aminoacridines containing various ring substituents, only ethidium was capable of promoting platinum binding to normally inaccessible DNA sequences.[150] Examination by ^{1}H NMR spectroscopy of the relative residence times of these compounds at their intercalation sites on DNA provided an explanation for their behavior.[150] Of the intercalators studied, ethidium has the longest mean residence time at its intercalation site by a factor of at least 6. Apparently, structural changes induced at a specific site by intercalation must persist long enough for platinum to approach and subsequently bind to the site. In the case of AO–Pt, the relatively short mean residence time of AO is offset by the advantageously positioned linked Pt atom near the DNA surface for potential coordination. Other as yet unexplored factors are important in specifying the ability of an intercalator to promote platinum binding, however. For instance, despite its slow DNA dissociation rate, the intercalator adriamycin does not promote platinum binding to inaccessible DNA sequences.

It has been suggested, on the basis of comparisons to X-ray crystallographic and NMR data, that the sequences excluding platinum binding may exhibit A-DNA-like structures.[125,150] The deep, narrow major groove of A-DNA (Figure 4) may sterically prohibit coordination of platinum to N7 of guanosine. Other types of structures that render N7 inaccessible for platinum binding are possible, however.

An interesting observation has been made recently concerning the reaction of *cis*-DDP with double-stranded DNA in the presence of ethidium bromide or proflavine.[126,151] After the mixture was incubated at 37 °C for 24 h, some of the bound ethidium or proflavine was resistant to extraction with butanol. Similar behavior was not observed for acridine orange, nor when *trans*-DDP was substituted for *cis*-DDP.[151] To explain these results, it was postulated that a ternary complex, the structure of which has not been elucidated, forms between *cis*-DDP, double-stranded nucleic acids, and ethidium or proflavine.[126] Adducts formed in the reaction of *cis*-DDP with a 275-base-pair restriction fragment of pBR322 in the presence or absence of ethidium were mapped by using the 3′ → 5′ exonuclease activity of T4 DNA polymerase.[126] Unlike similar experiments carried out using exonuclease III,[125,147] all d(GpG) dinucleotides, including one in a d(CpGpG) sequence, produced enzyme detectable *cis*-DDP binding sites in the absence of ethidium. Moreover, all but one d(ApG) sequence was modified by the platinum drug. As in the previous experiments,[125,147,148] however, new enzyme-detectable binding sites occurred for DNA platinated in the presence of ethidium. The somewhat different spectrum of adducts observed for *cis*-DDP binding to DNA as mapped by T4 DNA polymerase and exonuclease III may be due to different specificities of the two enzymes. A distorted structure detected by T4 DNA polymerase may go unnoticed by exonuclease III. For both enzymes, nuclease-detectable platinum binding sites may not be all of the binding sites that actually occur. Alternatively, the differences may be due to the regiospecificity of *cis*-DDP binding to restriction fragments of different sequences. More work is required to distinguish these possibilities.

The above results are interesting not only from a structural point of view but also from a clinical perspective. Combination chemotherapy of *cis*-DDP with intercalating drugs such as adriamycin often produces a synergistic effect.[2] The possible mutual influence of *cis*-DDP and other drugs on their DNA-binding properties provides, at the molecular level, a rationale for their chemotherapeutic synergism. It must be remembered, however, that in these experiments the D/N levels for both *cis*-DDP and intercalator are much higher than is likely to occur following clinical administration of the drugs. Further work is required to evaluate whether the observed synergism results from binding of *cis*-DDP and other drugs to similar regions of DNA in vivo.

D. Effects of Platinum Binding on DNA Structure

Not only are the site and mode of bidentate platinum binding to DNA dictated by local helix structure, but the resulting platinum adducts, in turn, distort the structure of the double helix. There has been much conflicting information, however, concerning the extent of the distortions caused by *cis*-DDP, *trans*-DDP, and even [Pt(dien)Cl]$^+$ on DNA structure.

Changes in both the UV[77,104,152] and circular dichroism[77] spectra upon binding of *cis*- or *trans*-DDP to DNA reflect a loss in normal base stacking within the helix. [Pt(dien)Cl]$^+$ does not seem to affect base stacking as measured by these techniques.[77] Increasing amounts of bound *cis*-{$Pt(NH_3)_2$}$^{2+}$ destabilize the DNA double helix, resulting in a decrease in melting temperature.[77,152–157] Most reports indicate that both *trans*-DDP and [Pt(dien)Cl]$^+$, however, stabilize DNA, raising its melting temperature.[77,152,153,156,157] It seems paradoxical that *trans*-DDP disrupts base stacking while simultaneously thermally stabilizing the DNA helix. Interstrand cross-linking and/or favorable hydrogen-bonding or electrostatic interactions may explain this behavior.

Platinum binding alters the structure of covalently closed, superhelical DNA plasmids, as measured by their mobility on electrophoresis gels.[54,158–160] Increasing amounts of both *cis*- and *trans*-DDP unwind the double helix, resulting in the removal of negative supercoils and, consequently, decreasing the electrophoretic mobility of the plasmid until the covalently closed DNA comigrates with relaxed circular DNA. While some studies have shown that equivalent amounts of bound

cis- and *trans*-DDP cause the same extent of DNA unwinding,[54,158] others have indicated that *cis*-DDP is a better unwinding agent than the trans isomer.[159,160] In the former studies, higher binding levels of both *cis*- and *trans*-DDP induced further positive supercoiling of covalently closed plasmids,[54,158] while in the latter studies only the cis isomer was observed to produce additional positive supercoiling.[159,160] The progressive reduction observed in unwinding angle as a function of increasing amounts of bound *cis*-DDP indicates that its major binding mode, the d(GpG) intrastrand cross-link, contributes to the unwinding phenomenon.[160]

The electrophoretic mobility of relaxed circular DNA increases upon platinum binding, presumably due to a shortening effect.[158] A dramatic decrease in the contour length of relaxed plasmid molecules with increasing amounts of bound platinum was revealed by electron microscopy.[77,148,158,161] Binding of platinum to plasmid DNA in the presence of ethidium bromide greatly inhibits the shortening effect.[20,148]

Long-range interstrand and/or intrastrand cross-linking may be responsible for platinum-induced diminution of the contour length of DNA.[77] To date, no experiments have been designed to evaluate this possibility. On the other hand, pronounced kinking of the DNA helix upon platinum binding could also cause these shortening effects. Some NMR results were interpreted on the basis of *cis*-$\{Pt(NH_3)_2\}^{2+}$-induced kinking in a double-stranded oligonucleotide.[162] The energies of both kinked and unkinked models of double-stranded oligonucleotide–*cis*-$\{Pt(NH_3)_2\}^{2+}$ adducts are comparable, as estimated from molecular mechanics calculations (vide infra).[163-165] Both long-range cross-linking and kinking are consistent with the inhibitory effects on shortening caused by ethidium. The stabilizing and stiffening influences of ethidium intercalation on the double helix would prohibit both structural distortions from occurring.

Although the importance of long-range DNA cross-linking by *cis*-DDP remains unknown, local distortions of DNA structure through the preferred binding modes discussed previously are significant. As discussed previously, such localized distortions block the normal action of restriction endonucleases,[107,108] exonuclease III,[109,110] T4 polymerase exonuclease activity,[126] and DNA polymerase.[70] Recently, site specifically platinated DNA was constructed by inserting a chemically synthesized and characterized dodecanucleotide containing one intrastrand *cis*-$[Pt(NH_3)_2\{d(GpG)\}]$ cross-link into the Hinc II restriction site of M13mp18 DNA (Figure 12).[166] The dodecanucleotide sequence was designed to have a unique Stu I restriction site at the position of the bound platinum. Additional restriction sites flank the site at which platinum is bound in this plasmid, as shown in Figure 12. The intrastrand platinum adduct prohibits Stu I from cutting at its normal recognition sequence. Upon removal of platinum with cyanide ion, Stu I sensitivity is restored, and the DNA is cleaved. The recognition sequence for Mae I overlaps the platinum cross-link by one nucleotide, sufficient for preventing cleavage by this restriction endonuclease. The restriction sites for Bam HI, Xba I, Pst I, and Sph I are farther removed from the site of platination, and these enzymes were unaffected. If any residual structural perturbations due to the uniquely

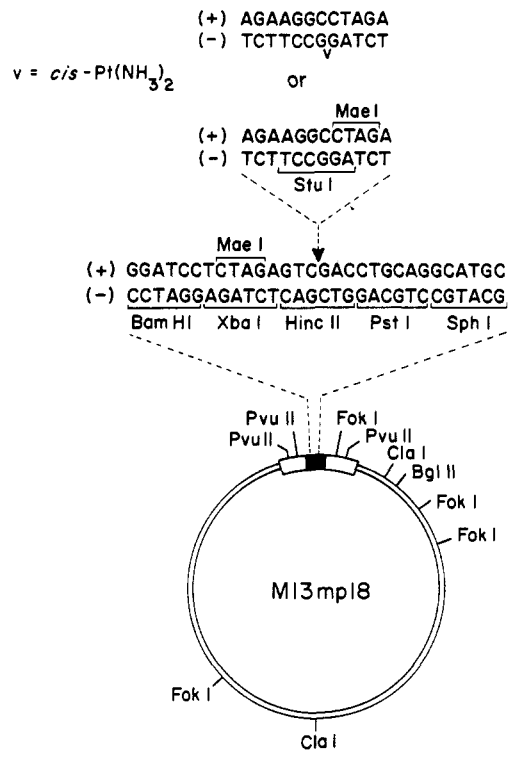


Figure 12. Map of the genome created by insertion of platinated or unplatinated d(TpCpTpApGpGpCpCpTpTpCpT)-d(ApGpApApGpGpCpCpTpApGpA) into the Hinc II site of M13mp18. Reproduced with permission from ref 166. Copyright 1986 American Chemical Society.

bound platinum occurs near these cleavage sites, they are not serious enough to inhibit enzymatic action.

The extent to which alterations in DNA structure induced by bound platinum produces single-stranded regions, where Watson–Crick hydrogen bonding is disrupted, is a matter of debate. Single-strand-specific nucleases cleave DNA treated with *cis*- or *trans*-DDP,[161,167,168] suggestive of duplex disruption. These nucleases may be sensitive, however, to a less severe distortion of the DNA helix than actual melting of the base pairs. Terbium(III) fluorescence was also used to probe unpaired guanosine residues and indicated the presence of melted regions upon binding of *cis*- and *trans*-DDP, but not the monofunctional reagent $[Pt(dien)Cl]^+$.[169] From both of these methods used to probe base pairing, it was concluded that *cis*-DDP perturbs DNA structure to a greater extent than *trans*-DDP.

On the other hand, differential-pulse polarographic studies indicated that low levels of bound *cis*-DDP and other platinum drugs do not create single-stranded regions, although a significant distortion of DNA structure was detected.[157] Single-stranded regions were detected by this technique when *trans*-DDP binds to DNA. Such a result is not unexpected if *trans*-DDP forms mainly intrastrand cross-links between two bases separated by one or more intervening nucleotides. Such binding would probably alter the structure of DNA to a greater degree than intrastrand cross-linking of two adjacent bases, as preferred by *cis*-DDP. Curiously, however, differential-pulse polarography studies also indicated that monofunctional $[Pt(dien)Cl]^+$ produces single-stranded regions.

NMR spectroscopic studies of double-stranded oligonucleotides containing the *cis*-$[Pt(NH_3)_2\{d(GpG)\}]$

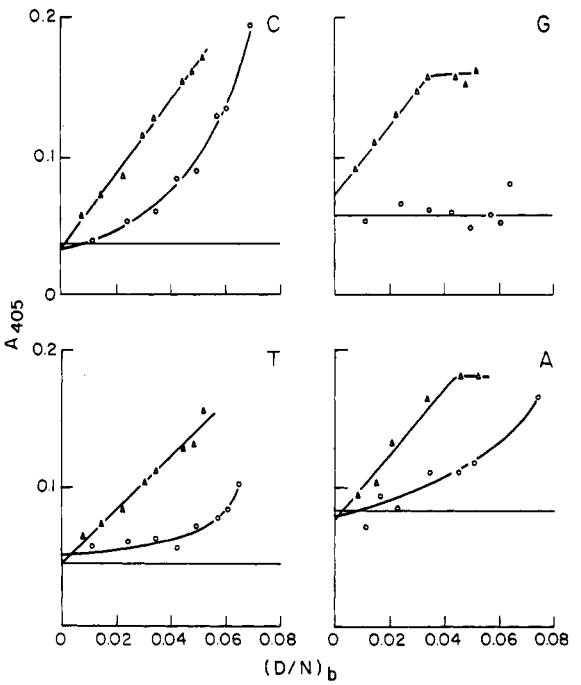


Figure 13. ELISA measurement of the binding of anti-cytidine (C), anti-guanosine (G), anti-thymidine (T), and anti-adenosine (A) antibodies to calf thymus DNA modified with increasing D/N of *cis*-DDP (○) and *trans*-DDP (△). Antibody binding was measured by absorbance 60 min after addition of the alkaline phosphatase substrate *p*-nitrophenyl phosphate. Background antibody binding to unmodified DNA is denoted by the solid horizontal line. Data points measured in triplicate varied an average of $\pm 0.007 A_{405}$ unit from their mean. Reproduced with permission from ref 174. Copyright 1986 American Chemical Society.

adduct have provided evidence that, at least at low temperature, base pairing is possible,[170-172] although not necessarily of the normal, Watson–Crick type (vide infra). At 25 °C, and an added D/N ratio of 0.1, reaction of poly(I)·poly(C) with $[Pt(en)Cl_2]$ in H_2O causes a loss in the intensity of the N1-imino inosine resonance in the proton NMR spectrum.[173] This result is consistent with a weakening of the double helix and faster exchange of this proton with water. Moreover, the ^{31}P NMR resonance of the poly(C) strand sharpens upon platination of the homocopolymer, and its chemical shift corresponds closely to that of single-stranded poly(C).[173] This result is also consistent with base-pair disruption. No such structural modifications were observed in the cases of *trans*-DDP or $[Pt(dien)Cl]^+$.[173]

Antibodies raised against protein-conjugated nucleosides have also been used to probe the structures of calf thymus DNAs modified by *cis*-DDP, *trans*-DDP, and $[Pt(dien)Cl]^+$.[174] These anti-nucleoside antibodies bind well to denatured but not to native DNA and are therefore useful agents for studying the extent and specificity of DNA base-pair disruptions in vitro. Modification of DNA with increasing levels of bound *cis*-DDP increases in a nonlinear fashion the binding of antibodies specific for adenosine, thymidine, and cytidine. At low levels of platination, anti-cytidine antibodies bind best to platinated DNA and, at higher levels of modification, anti-adenosine and anti-thymidine antibodies bind as well (Figure 13). No significant increase of binding was observed for anti-guanosine antibodies to DNA modified by *cis*-DDP. In contrast, binding of all four anti-nucleoside antibodies increased in a linear fashion to DNA modified by low levels of *trans*-DDP. Moreover, at equivalent levels of bound platinum, all four antibodies reacted more with DNA modified by *trans*-DDP than with DNA modified by *cis*-DDP. There was no change in anti-nucleoside antibody binding to DNA modified with $[Pt(dien)Cl]^+$ compared to unmodified DNA, up to D/N ratios of 0.12. From these results, it is apparent that binding of both of the bifunctional platinum complexes, *cis*-DDP and *trans*-DDP, alters the structure of calf thymus DNA, exposing the nucleoside bases, while binding of the monofunctional complex $[Pt(dien)Cl]^+$ does not. The results are consistent with coordination of *cis*-DDP primarily at guanosine residues, which exposes cytidines in the opposite strand for binding to the antibodies. Coordination of *cis*-DDP at adjacent guanosines prohibits binding by the anti-guanosine antibodies. This conclusion was substantiated by an experiment demonstrating that the dinucleoside monophosphate d(GpG) competitively inhibits binding of anti-guanosine antibodies to denatured DNA, whereas its *cis*-DDP adduct, *cis*-$[Pt(NH_3)_2\{d(GpG)\}]^+$, does not. The effects of *trans*-DDP binding to DNA on anti-nucleoside antibody recognition are consistent with the formation of 1,3-intrastrand cross-links, which are expected to disrupt the double helix to a greater extent than adducts formed by *cis*-DDP. The greater disruption observed for low levels of bound *trans*-DDP vs. *cis*-DDP offers a structural rationale for the greater apparent repair of *trans*-DDP-platinated DNA in mammalian cell lines.[32,35]

V. Structural Studies of Specific Pt–DNA Adducts

A. Comparison of Techniques

Recently, structural details of intrastrand platinum–DNA adducts have become available through NMR spectroscopy and X-ray diffraction studies. Although some of this work has been carried out on platinum complexes of naturally occurring nucleic acids,[131,175-177] most structural information has been derived from platinum complexes with short, synthetic oligonucleotides. These materials are now relatively easy to obtain thanks to the advent of new techniques for synthesizing milligram quantities of oligonucleotides having defined sequences and finite lengths.[178,179]

Most of the structural work on platinum–DNA adducts has employed NMR spectroscopy. The NMR spectra of these platinated oligonucleotides are typically recorded in D_2O, where the nonexchangeable base and deoxyribose sugar proton resonances are readily observed (Figure 14). Platinum binding sites are usually determined by monitoring the pH dependence of the nonexchangeable base proton chemical shifts. Protonation and deprotonation reactions of the heteroatoms on the bases affect the environments of nonexchangeable protons, resulting in changes in their chemical shifts. For example, since binding of platinum to N7 of guanosine lowers the pK_a for deprotonation at N1 by approximately 2 units, and blocks protonation at N7, changes are produced in the pH-dependent chemical shift of guanosine H8, as seen in Figure 15. Spectral assignments and extraction of structural information are achieved by various additional strategies, including decoupling and NOE techniques.[180]

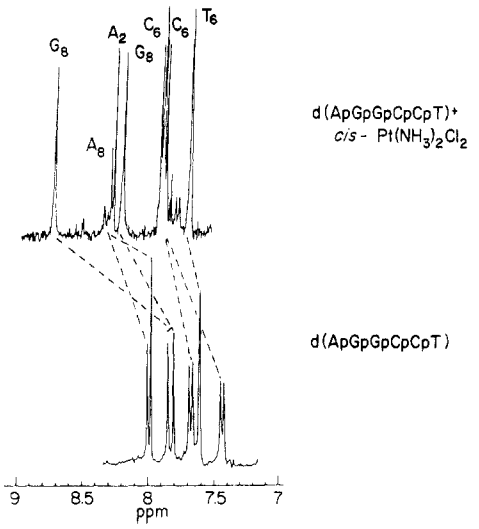


Figure 14. Downfield regions of 300-MHz ^1H NMR spectra (9.0–5.0 ppm) of $[d(ApGpGpCpCpT)]_2$ (3.5 mM duplex, 35 °C, no buffer, pH* 6.9) and its *cis*-DDP adduct (2.5 mM strand, 70 °C, no buffer, pH* 6.7) in D_2O, revealing the nonexchangeable base proton resonances. The pyrimidine resonances of the latter sample show no chemical shift changes with temperature over the range $35 < T < 70$ °C, while the purine resonances show a slight temperature-dependent chemical shift change of up to 0.1 ppm. The symbol pH* signifies uncorrected meter readings for samples in D_2O. Reproduced with permission from ref 114. Copyright 1982 American Chemical Society.

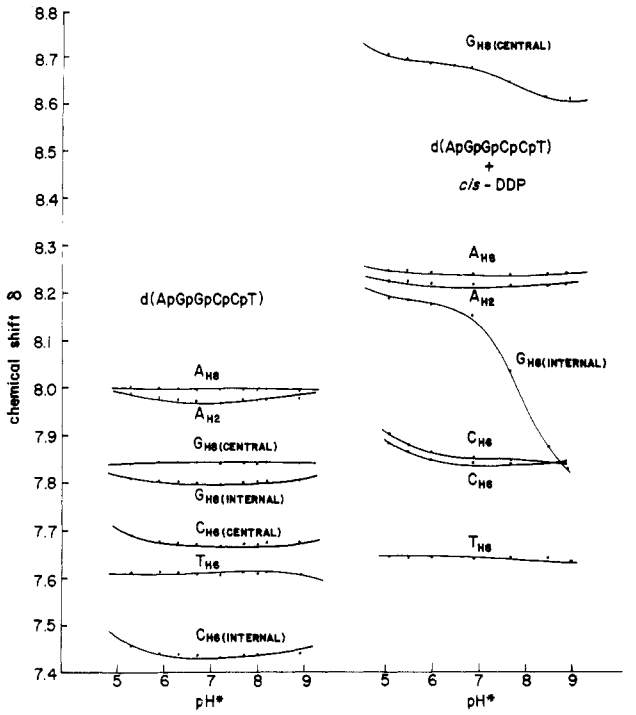


Figure 15. Chemical shift (δ) vs. pH* of the nonexchangeable base protons of $[d(ApGpGpCpCpT)]_2$ and its *cis*-DDP adduct. Conditions are the same as in Figure 14. Adopted from ref 114.

On the other hand, very few single-crystal X-ray diffraction structures of platinum–nucleic acid complexes have been reported. The success rate for crystallizing nucleic acids is similar to that for proteins. Experience has indicated that only about 1 out of 10 random, self-complementary sequences yields single crystals.[181] Whether the addition of a platinum atom renders the challenge more or less difficult is unknown. Certainly if diffracting crystals are obtained, the subsequent solution of the structure is facilitated by the presence of a heavy metal atom. In order to circumvent direct crystallization of platinated oligonucleotides, some researchers have attempted to gain structural information by soaking *cis*-DDP into existing nucleic acid crystals, either yeast $tRNA^{Phe}$ [131,175] or a B-DNA dodecamer.[182] The loss of crystal order observed upon drug binding in these soaking experiments is consistent with perturbation of the native DNA or RNA structures. Thus, only limited information was gained (see following section), and it seems unlikely at present that high-resolution geometric details can be obtained by this method. A preferred, although perhaps more difficult, approach for obtaining geometric data is to synthesize and purify the platinum–nucleic acid complex prior to crystallization. Only two compounds, both containing single-stranded oligonucleotides, have thus far been successfully studied by X-ray diffraction using this strategy (vide infra).

One advantage of NMR over X-ray crystallographic techniques for studying the conformations of Pt–DNA adducts is that structural information is obtained in solution, the natural state of DNA in biology. Moreover, details about the relative conformational flexibilities of oligonucleotides can often be obtained. Metrical parameters, however, such as bond distances, precise torsion angles, and the dihedral angles between purine and pyrimidine base planes, are more difficult or even impossible to procure by NMR. Moreover, as the size of the molecule increases, it becomes harder to obtain detailed conformational information, owing to the complexity of the NMR spectrum. The same limitation applies to X-ray crystallographic investigations of biopolymers, but to a far lesser degree. A possible disadvantage of using X-ray diffraction is that the structure of the molecule studied may be influenced by crystal-packing forces, although the high water content of many crystals of biopolymers partially obviates this problem. Clearly, both NMR and X-ray techniques complement one another and, together, yield much experimental insight into the structures of platinum–DNA adducts. Some theoretical insight into the possible structures of such adducts has been afforded recently by molecular mechanics calculations.[163–165] The calculations have been carried out on both single- and double-stranded oligonucleotides, providing some useful comparisons.

B. Soaking of *cis*- and *trans*-DDP into Preformed DNA or RNA Crystals

When *cis*-DDP was soaked into orthorhombic crystals of $tRNA^{Phe}$ in buffer containing 10 mM chloride ion, platinum atoms were found at two sites.[175] Both platinum atoms bind monofunctionally to N7 atoms of isolated guanosine residues. Under the same conditions, *trans*-DDP also binds monofunctionally, but at four different sites. One of these four sites appeared to be N1 of adenosine, and the remaining sites were guanosine N7 atoms. Although the platinum ligands could not be identified, it was suggested that the fourth ligands on both monofunctionally bound platinum atoms in the *cis*-DDP case are water molecules, hydrogen bonded to oxygen atoms in the tRNA structure.[175] Since water is an excellent leaving group, it would be surprising if it were not displaced by a donor atom from the nucleic acid. As was subsequently recognized,[131] it

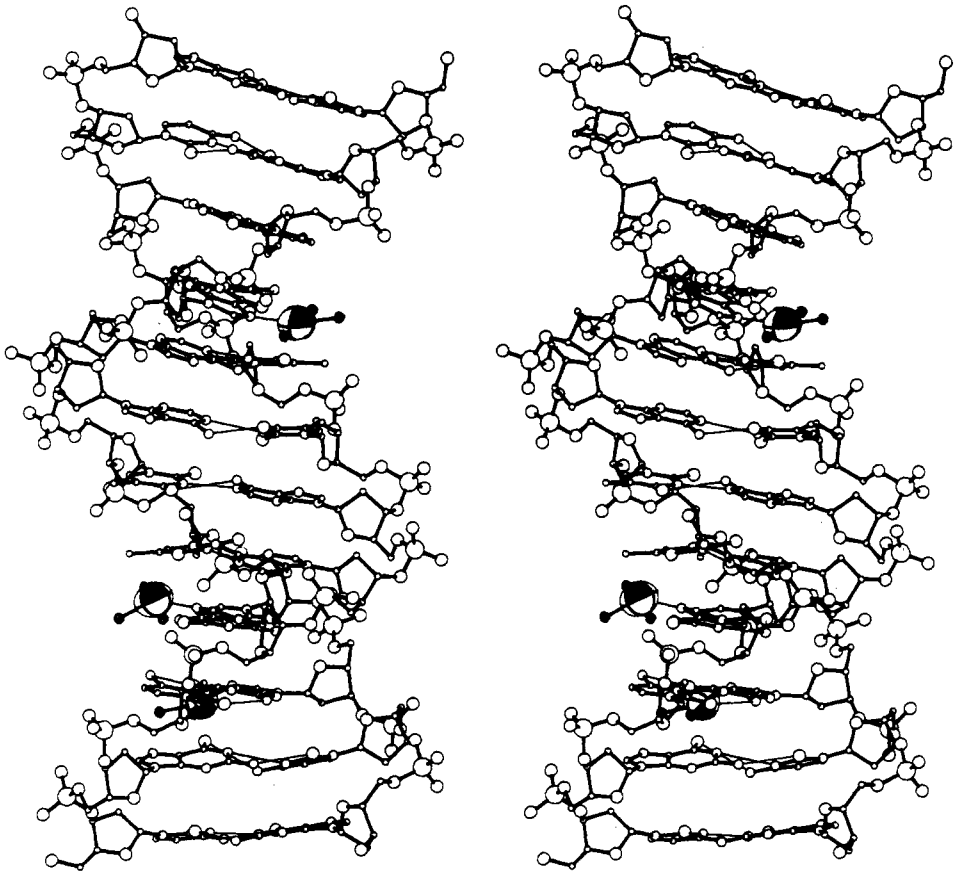


Figure 16. View of one cisplatin complex of duplex d(CpGpCpGpApApTpTpCpGpCpG). Base pair C(1)·G(24) is at the top, and G(12)·G(13) is at the bottom. Pt atoms at guanosines G(4), G(16), and G(10) (from top to bottom) are represented by their anisotropic thermal ellipsoids. The three smaller crossed spheres around each Pt are ligand sites obtained as described in ref 182. Reproduced with permission from ref 182. Copyright 1982 European Molecular Biology Organization.

is most likely that, under these conditions, the fourth ligand is chloride, which would better explain the observed monofunctional binding.

cis-DDP also binds monofunctionally when soaked into crystals of the self-complementary, double-stranded B-DNA dodecamer d(CpGpCpGpApApTpTpCpGpCpG).[182] Platinum appears to bind three of the eight guanosine N7 atoms contained in the double helix for three crystals that were studied. Only partial occupancy of platinum was attained at these sites, precluding detailed geometric information. All attempts to achieve complete substitution resulted in severe loss of crystal order. In all three of the crystals, the preferred platinum binding sites were G16 > G4 > G10 (Figure 16). This result supports the idea that local sequence will dictate the *cis*-DDP binding preferences on DNA, as discussed above. Platinum binding shifts the modified base pairs relative to their positions in the unmodified structure out of the stack toward the platinum atoms in the major groove.

In the only experiment of its kind that resulted in bidentate platinum binding, hydrolysis products of *cis*-DDP were soaked into monoclinic crystals of $tRNA^{Phe}$.[131] Reaction with the drug physically degraded the crystals, resulting in low resolution (~6-Å) data. Electron density assigned to platinum was observed at specific sites in $tRNA^{Phe}$, but no geometrical conclusions could be drawn. Platinum binding occurs at (GpG), (ApG), and (CpG) sites. In the last, cytosine is believed to undergo a rotation to allow N3 to coordinate. An elongated region of electron density occurred at a (GpGpApG) sequence. Platinum binding to more than one site in this sequence seems probable.

C. Intrastrand *cis*-Diammineplatinum(II) Cross-Link of Adjacent Guanosines

1. Single-Stranded Adducts

To date, most structural studies of platinated oligonucleotides have been concerned with the d(GpG) intrastrand cross-link, the major adduct formed between *cis*-DDP and DNA (Table III).[112-115,162,170-172,183-192] In every such sequence studied so far, the platination reaction results in one main product, a single-stranded complex in which platinum coordinates to the N7 atoms of two adjacent guanosines. The self-complementary sequences d(CpCpGpG) and d(ApGpGpCpCpT) were allowed to react with *cis*-DDP at temperatures where the oligonucleotides are largely double stranded.[114,186] For example, under conditions of 3.5 mM oligonucleotide, 5 mM phosphate, and 1 mM EDTA, the duplex molecule $[d(ApGpGpCpCpT)]_2$ melts noncooperatively. The terminal A-T base pairs are frayed at approximately 37 °C, while the GpGpCpC core remains intact until the melting temperature of 60 ± 3 °C is reached.[114] At 37 °C, the temperature at which its reaction with *cis*-DDP was carried out, the oligonucleotide is mainly duplex. Interestingly, regardless of the duplex oligonucleotide to platinum ratios or concentrations employed, only the single-stranded adduct was obtained.[191] At a duplex to platinum ratio of 1:1, this adduct and unreacted oligonucleotide accounted for

TABLE III. Platinum–Oligonucleotide Complexes Containing the Main Adducts Formed between *cis*- and *trans*-DDP and DNA or RNA as Studied by NMR Spectroscopy and X-ray Crystallography

	technique	ref
cis-[Pt(NH$_3$)$_2$(GpG)] Adducts		
cis-[Pt(NH$_3$)$_2$(GpG)]	NMR	112, 113
cis-[Pt(NH$_3$)$_2${d(GpG)}]	NMR	113, 183
cis-[Pt(NH$_3$)$_2${d(pGpG)}]	NMR, X-ray	113, 189
cis-[Pt(NH$_3$)$_2${d(CpGpG)}]	NMR, X-ray	184, 190
cis-[Pt(NH$_3$)$_2${d(CpCpGpG)}]	NMR	185, 186
cis-[Pt(NH$_3$)$_2${d(ApTpGpG)}]	NMR	187, 188
cis-[Pt(NH$_3$)$_2${d(ApGpGpCpCpT)}]	NMR	114
cis-[Pt(NH$_3$)$_2${d(TpGpGpCpCpA)}]	NMR	155
cis-[Pt(NH$_3$)$_2${d(CpCpApTpGpG)}]	NMR	187
cis-[Pt(NH$_3$)$_2${d(GpApTpCpCpGpGpC)}][a]	NMR	171
cis-[Pt(NH$_3$)$_2${d(TpCpTpCpGpGpTpCpTpC)}][a]	NMR	162, 170
cis-[Pt(NH$_3$)$_2${d(GpCpCpGpGpApTpCpGpC)}][a]	NMR	172
cis-[Pt(NH$_3$)$_2$(ApG)] Adducts		
cis-[Pt(NH$_3$)$_2$(ApG)]	NMR	128
cis-[Pt(NH$_3$)$_2${d(GpApG)}]	NMR	129
cis-[Pt(NH$_3$)$_2$(GNG)] Adducts		
cis-[Pt(NH$_3$)$_2${d(GpCpG)}]	NMR	202, 205
cis-[Pt(NH$_3$)$_2${d(pCpGpCpG)}]	NMR	186
cis-[Pt(NH$_3$)$_2${d(pGpCpGpC)}]	NMR	186
cis-[Pt(NH$_3$)$_2${d(CpGpCpG)}]	NMR	186
cis-[Pt(NH$_3$)$_2${d(GpTpG)}]	NMR	203
cis-[Pt(NH$_3$)$_2${d(GpApG)}]	NMR	129
cis-[Pt(NH$_3$)$_2${d(TpCpTpCpGpTpGpTpCpTpC)}][a]	NMR	204
trans-[Pt(NH$_3$)$_2$(GNG)] or -[Pt(NH$_3$)$_2$(ANG)] Adducts		
trans-[Pt(NH$_3$)$_2${d(GpTpG)}]	NMR	203
trans-[Pt(NH$_3$)$_2${d(GpCpG)}]	NMR	206
trans-[Pt(NH$_3$)$_2${d(ApGpGpCpCpT)}]	NMR	207

[a] For these sequences, double-stranded platinated oligonucleotide complexes were also studied.

greater than 95% of the total optical density at 254 nm. There was no evidence either for interstrand cross-linking or formation of an intrastrand cross-linked duplex oligonucleotide.[191] Intermediates were observed during the reaction of d(TpGpGpCpCpA) with *cis*-[Pt(NH$_3$)$_2$Cl$_2$], which subsequently converted to the single-stranded product.[115]

These results indicate that coordination of *cis*-{Pt(NH$_3$)$_2$}$^{2+}$ to adjacent guanosines destabilizes the double helix. At added self-complementary duplex to platinum ratios of 1:1, a product mixture consisting of platinated single-stranded and unmodified double-stranded oligonucleotides is thermodynamically more stable than the corresponding platinated double-stranded oligonucleotides. The syntheses of platinated double-stranded oligonucleotides has been achieved only by using non-self-complementary oligonucleotides (vide infra).

Features of the local structure of the platinated d(GpG) intrastrand cross-link in solution appear to be independent of the oligonucleotide sequence in which it is embedded. In the syn conformation of guanosine, the deoxyribose H1′ proton is much closer to guanine H8 than in the anti conformation (Figure 6). Complete exchange of the guanosine H8 atoms of *cis*-[Pt(NH$_3$)$_2$(GpG)] for deuterium was accompanied by only a 20% increase of the T_1 relaxation times of both H1′ protons, indicating the anti,anti configuration.[113] Subsequent NOE experiments on *cis*-[Pt(NH$_3$)$_2${d(ApTpGpG)}][188] and *cis*-[Pt(NH$_3$)$_2${d(CpGpG)}][184] confirmed that, in solution, the (GpG)–platinum adduct has an anti,anti configuration of the two guanine base planes. In these platinated sequences, an NOE has been observed between the two guanosine (or inosine) H8 atoms, reflecting a head-to-head orientation.

Further details of the solution structure of this adduct were afforded by NMR conformational analyses of *cis*-[Pt(NH$_3$)$_2${d(GpG)}],[183] *cis*-[Pt(NH$_3$)$_2${d(CpGpG)}],[184] and *cis*-[Pt(NH$_3$)$_2${d(ApTpGpG)}].[188] Assignments of the ^{1}H NMR spectra of these compounds were achieved by extensive decoupling experiments. For the former two molecules, chemical shifts and coupling constants were extracted from the data by computer simulations. Previous work had revealed an unusual splitting pattern for one of the two guanosine H1′ resonances, consistent with a near-zero value for the $J_{H_1'H_2'}$ coupling constant.[113] This result usually signifies that the deoxyribose ring has a C3′-endo (N) conformation. Although some of the nucleotides in crystals of B-DNA have C3′-endo sugar puckers,[145] this hydrated form of DNA is generally characterized by C2′-endo sugar puckers (Figure 6).[8] The C3′-endo conformation, more commonly found in polycrystalline fibers and single crystals of A-DNA and in RNA,[8] substantially diminishes the distance between adjacent backbone phosphorus atoms. NMR results for platinated oligonucleotides reveal that, at any given instant, 100% of the 5′-guanosine sugar rings in *cis*-[Pt(NH$_3$)$_2${d(GpG)}] and *cis*-[Pt(NH$_3$)$_2${d(CpGpG)}] and >91% of those in *cis*-[Pt(NH$_3$)$_2${d(ApTpGpG)}] are found in a C3′-endo conformation. The sugar conformations of the 3′-guanosines in these same molecules are more flexible, with 70–80% of the molecules in the C2′-endo (S) conformation, depending on temperature. Both sugars of the (GpG) moiety of the corresponding unplatinated oligonucleotides are mainly in a C2′-endo conformation.

From the above data and the torsion angle population distributions in Table IV, the 3′-end of *cis*-[Pt(NH$_3$)$_2$-{d(GpG)}] was judged to be somewhat more flexible than the 5′-end.[183] From the ^{1}H scalar coupling con-

TABLE IV. Population Distributions of Backbone Conformation Angles γ and β for *cis*-[Pt(NH$_3$)$_2$\{d(GpG)\}][183]

torsion angle	conformer	temp, °C	% of conformer
γ(1)[a]	g$^+$	23	77
		53	72
		81	70
γ(2)[a]	g$^+$	23	79
		53	67
		81	65
β(2)[a]	t	23	93
		53	92
		81	89

[a] Key: (1) refers to the 5′-end of the adduct; (2) refers to the 3′-end of the adduct.

stants, torsion angles were computed for the most abundant conformer of *cis*-[Pt(NH$_3$)$_2$\{d(GpG)\}] at room temperature (Table V). Conformations for the phosphodiester backbone torsion angles ζ(1) and α(2) were obtained from ^{31}P NMR chemical shift data. Similar results were obtained for the platinated portion of *cis*-[Pt(NH$_3$)$_2$\{d(CpGpG)\}].[184]

X-ray crystallographic data have afforded even more detail about the local structure of the bifunctional d(GpG) adduct.[189,190] Table VI summarizes X-ray crystallographic information derived from X-ray diffraction studies of two platinated oligonucleotides. The X-ray structure of *cis*-[Pt(NH$_3$)$_2$\{d(pGpG)\}] consists of an aggregate of four crystallographically independent molecules linked by a network of intermolecular base–base stacking and hydrogen-bonding interactions.[189] The study thus provides four crystallographically independent views of the molecule (molecules 1–4). Specifically, two conformationally distinct classes occur that are related by a noncrystallographic twofold axis. The first class consists of molecules 1 and 2, and the second class, of molecules 3 and 4. Similarly, three crystallographically independent molecules comprise the crystal lattice of *cis*-[Pt(NH$_3$)$_2$\{d(CpGpG)\}] (molecules 1–3).[190] Two such molecules (1 and 2) form a hydrogen-bonded dimer of dimers, and the third is hydrogen-bonded to a symmetry-related molecule. The sugar–phosphate backbone of molecule 3 apparently undergoes a large amount of thermal motion, and its detailed conformation cannot yet be determined accurately.

The molecular structure of cis-[Pt(NH$_3$)$_2$\{d(pGpG)\}] is presented in Figure 17. The square-planar environment of the platinum atom is comprised of two ammine ligands and two N7 atoms of guanosines from the same strand. The Pt–N7 bond lengths are normal, ~2.0 Å. In double-helical, stacked DNA structures,

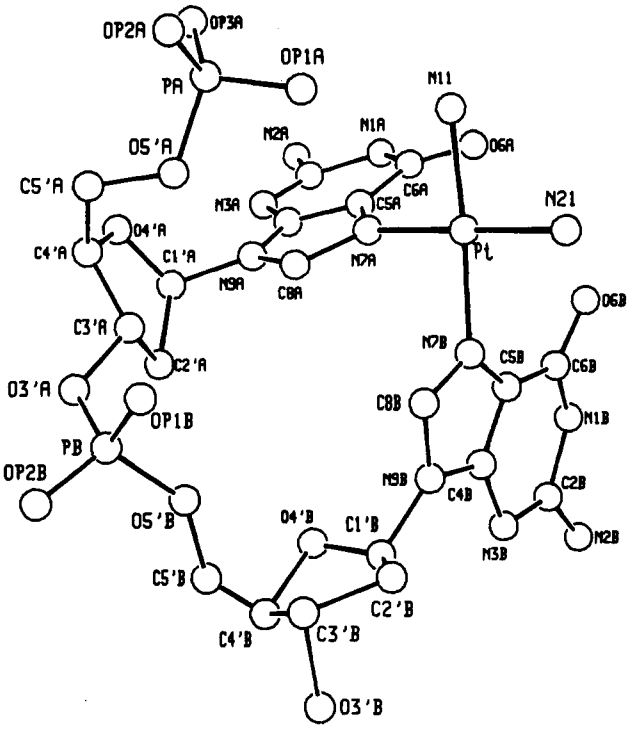


Figure 17. Molecular structure of one of the four molecules from the X-ray structure of *cis*-[Pt(NH$_3$)$_2$\{d(pGpG)\}]. Reproduced with permission from ref 189. Copyright 1985 Science.

base planes are nearly parallel to one another.[8] Formation of the intrastrand cross-link forces open the dihedral angles between the guanosine base planes to values ranging from 76 to 87° in both X-ray structures, completely disrupting base–base stacking interactions.[189,190] Platinum deviates by less than 0.4 Å from the guanosine base planes in molecules 1–4 of *cis*-[Pt(NH$_3$)$_2$\{d(pGpG)\}] and in molecules 1 and 2 of *cis*-[Pt(NH$_3$)$_2$\{d(CpGpG)\}]. A head-to-head orientation of the two guanine rings occurs, with both O6 atoms on the same side of the platinum coordination plane.

Table V contains information about the backbone torsion angles and sugar geometries of *cis*-[Pt(NH$_3$)$_2$\{d(GpG)\}] adducts, derived from both solid-state and solution structural studies. Many similarities are seen among the different structures. The pseudorotation angles, *P*, of the 5′-deoxyribose rings all fall between −12 and +27°, characteristic of a C3′-endo or C2′-exo conformation. These angles result in phosphorus···phosphorus distances of 5.5–6.2 Å, the A-DNA range.[8] Conformations of the 3′-sugars are diametrically opposed on the pseudorotation cycle, with C2′-endo or C1′-exo sugar puckers and corresponding *P* values be-

TABLE V. Backbone and Deoxyribose Torsion Angles for *cis*-[Pt(NH$_3$)$_2$(GpG)] from Solid-State and Solution Studies[a]

	γ(1)	δ(1)	ε(1)	ζ(1)	*P*(1)	Ψ(1)	α(2)	β(2)	γ(2)	δ(2)	*P*(2)	ψ(2)
molecule 1[b]	56 (4)	94 (4)	−142 (3)	−65 (3)	−12	33	−85 (8)	−143 (6)	30 (11)	147 (7)	84	38
molecule 2[b,c]	65 (4)	104 (4)	−144 (3)	−64 (3)	−8	35	−77 (6)	−141 (4)	84 (16)	108 (15)	138	28
molecule 3[b]	55 (4)	91 (5)	−126 (4)	−71 (5)	23	46	−57 (7)	−168 (6)	47 (8)	150 (7)	130	49
molecule 4[b]	36 (6)	101 (5)	−128 (4)	−69 (4)	27	48	−49 (5)	−161 (4)	42 (7)	137 (6)	136	43
molecule 1[d]	48 (5)	90 (3)	−148 (4)	−50 (5)	14	34	−112 (7)	−197 (5)	99 (10)	107 (3)	151	42
molecule 2[d]	53 (4)	97 (3)	−132 (4)	−64 (4)	−12	34	−60 (6)	−173 (4)	46 (5)	127 (4)	133	42
NMR data[e]	52	87 (3)	−162 (4)	g$^-$	−1 (7)	38 (2)	g$^-$	~180	50–60	137 (2)	149 (2)	34 (2)

[a] Torsion angles are defined in Figure 5 and are tabulated in degrees. *P* and and ψ are defined in section IV. Key: (1) refers to the 5′-end of the adduct; (2) refers to the 3′-end of the adduct. [b] Refers to one of the four crystallographically independent molecules in the X-ray structure of *cis*-[Pt(NH$_3$)$_2$\{d(pGpG)\}].[189] [c] A second conformation has been identified in further modeling the electron density in the region of this disordered molecule[192] and will be reported in more detail elsewhere. [d] Refers to one of the three crystallographically independent molecules in the X-ray structure of *cis*-[Pt(NH$_3$)$_2$\{d(CpGpG)\}].[190] Values for molecule 3 were excluded (see text). [e] From NMR solution study of *cis*-[Pt(NH$_3$)$_2$\{d(GpG)\}].[183]

TABLE VI. X-ray Crystallographic Data for Two *cis*-Diammineplatinum(II) Adducts of d(GpG)-Containing Oligonucleotides

	cis-[Pt(NH_3)$_2$-{d(pGpG)}][a]	*cis*-[Pt(NH_3)$_2$-{d(CpGpG)}][b]
unit cell		
a, Å	31.326	25.632
b, Å	35.679	53.848
c, Å	19.504	27.465
V, Å^3	21 799	37 907
space group	$P2_12_12$	$C222_1$
resolution, Å	0.94	1.8
refinement	unconstrained least squares	constrained least squares
current *R* factor, %	8.4	14.3

[a] From ref 189. [b] From ref 190.

TABLE VII. Glycosidic Torsion Angles, χ (deg), for *cis*-[Pt(NH_3)$_2$d(GpG)] Adducts from X-ray Structural Work

	5′χ (C3′-endo sugar pucker)	3′χ (C2′-endo sugar pucker)	Δχ[c]
molecule 1[a]	−94	−93	−1
molecule 2[a]	−89	−110	21
molecule 3[a]	−138	−117	−21
molecule 4[a]	−142	−127	−15
molecule 1[b]	−69	−116	47
molecule 2[b]	−77	−91	14
molecule 3[b]	−73	−120	47

[a] From the X-ray structure of *cis*-[Pt(NH_3)$_2$d(pGpG)].[189] [b] From the X-ray structure of *cis*-[Pt(NH_3)$_2$d(CpGpG)].[190] [c] Δχ = 5′χ − 3′χ.

tween 84 and 151°. In the structure of *cis*-[Pt(NH_3)$_2$-{d(pGpG)}], large thermal parameters and some unusual bond lengths were observed for the 3′-sugars, indicative of substantial thermal motion and/or disorder.[189] These features are reminiscent of the relative conformational flexibility observed at the 3′-end of *cis*-[Pt(NH_3)$_2$d-(GpG)] in solution. Restrained least-squares refinement of the structure restored the somewhat disparate *P*(2) value of 84° in molecule 1 to a value in closer agreement with those in molecules 2–4.[192]

The backbone torsion angles α–ζ fall near or within the ranges of values seen in crystallographic studies of helical A- and B-type DNAs.[145,193–195] One apparent deviation is that of α(2) in molecule 1 of the structure of *cis*-[Pt(NH_3)$_2$d(CpGpG)]. Its value of −112 (7)° is somewhat unusual, even for nonhelical structures,[8] and may simply reflect the relatively low resolution of this structure determination. Moreover, additional refinement[192] of the *cis*-[Pt(NH_3)$_2$d(pGpG)] structure revealed disorder of the C4′ and C5′ atoms of the 3′-sugar residue of molecule 2 over two positions. One of the resulting conformers has α(2) and γ(2) values deviating significantly from standard A- and B-type DNA structures.

Further insight into the perturbations of normal, helical DNA due to coordination of *cis*-{Pt(NH_3)$_2$}$^{2+}$ can be gained by examining the values for the glycosyl angle χ (see section IVA). The χ values of the (GpG) segment of all of the molecules in both X-ray structures (Table VII) fall either within the anti (−91 to −142°) or high-anti (−69 to −89°) range.[189,190] In crystals of B-DNA, where a broad range of sugar puckers and, consequently, of values for torsion angle δ occur, χ is correlated with δ.[196] For high values of δ (~145°) typical of C2′-endo sugar puckers, χ is around −100°. For lower values of

TABLE VIII. Base/PtN_4 Dihedral Angles (deg) and Ammine (N)• • •Guanine (O6) Distances (Å) from the X-ray Structures of *cis*-[Pt(NH_3)$_2$d(GpG)] Adducts

	5′-Gua/PtN_4	3′-Gua/PtN_4	O6···NH_3
molecule 1[a]	110	86	3.33
molecule 2[a]	111	85	3.50
molecule 3[a]	80	58	3.11
molecule 4[a]	77	60	3.16
molecule 1[b]	127	90	2.97
molecule 2[b]	130	90	2.94
molecule 3[b]	124	87	3.03

[a] From the X-ray structure of *cis*-[Pt(NH_3)$_2$d(pGpG)].[189] [b] From the X-ray structure of *cis*-[Pt(NH_3)$_2$d(CpGpG)].[190]

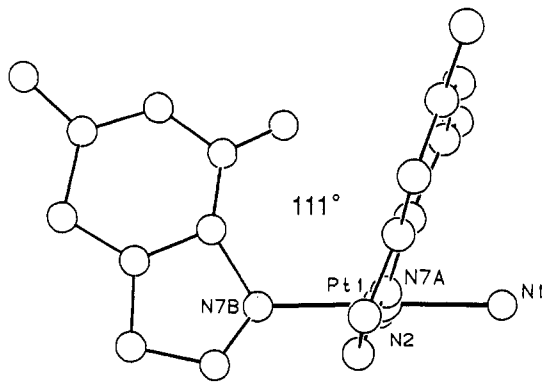


Figure 18. Diagram of molecule 1 of *cis*-[Pt(NH_3)$_2$d(pGpG)], illustrating the convention for measuring base/PtN_4 dihedral angles as described in the text. Atoms of the platinum coordination plane are labeled. The deoxyribose phosphodiester backbone is omitted for clarity. The angle between the 5′-base (N7A) and PtN_4 is 111°.

δ (~90°), typical of C3′-endo sugar puckers, χ decreases to around −140°, resulting in optimal base–base stacking interactions. Inspection of χ and δ values in Table VII for the X-ray structures of *cis*-[Pt(NH_3)$_2$d(pGpG)] and *cis*-[Pt(NH_3)$_2$d(CpGpG)] reveals that the B-DNA correlation rule does not apply. Instead, broad ranges of values exist for these two angles, indicating substantial conformational freedom about both glycosyl bonds, even though the 5′-sugar is locked into a C3′-endo form. Moreover, the various differences between χ values for 5′- and 3′-nucleosides in each molecule (Table VII) further reflect conformational flexibility.

Considerable variation also occurs in dihedral angles between platinum coordination and guanine base planes (Table VIII). According to convention,[197] these PtN4/base dihedral angles are assigned by placing the Pt–N7 bond of the base associated with the dihedral angle of interest perpendicular to, and projecting out of, the plane of the paper. The opposite base is positioned to the left of the diagram, as in Figure 18. For molecules 1–4 of *cis*-[Pt(NH_3)$_2$d(pGpG)], these angles occur in pairs, reflecting the approximate twofold symmetry of the crystal lattice. The relatively small 3′-Gua/PtN_4 angles of molecules 3 and 4 result in intramolecular ammine (N)···guanosine (O6) distances of 3.1–3.2 Å, which may signify a very weak hydrogen bond. In the structure of *cis*-[Pt(NH_3)$_2$d(CpGpG)], the 3′-Gua/PtN_4 angles are similar to those in molecules 1 and 2 of *cis*-[Pt(NH_3)$_2$d(pGpG)]. The 5′-Gua/PtN_4 angles in *cis*-[Pt(NH_3)$_2$d(CpGpG)] are much more pronounced, however, giving rise to weak ammine (N)···guanosine (O6) hydrogen bonds with distances of ~2.95 Å (Table VIII).

Intramolecular hydrogen bonding between a coordinated ammine and an oxygen atom of the 5′-phosphate group occurs in at least three out of four molecules in the solid-state structure of *cis*-[$Pt(NH_3)_2${d(pGpG)}].[189] For molecules 1–4, the N···O distances are 2.72 (5), 2.83 (3), 3.03 (3), and 3.22 (5) Å, respectively. Evidence for ammine (H)···phosphate (O) hydrogen bonding in solutions of AMP–platinum adducts has been recently found.[132] In the X-ray structure of *cis*-[$Pt(NH_3)_2${d(CpGpG)}], no such hydrogen bonding was observed.[190] The orientations of the 5′-cytosine bases, which participate in intermolecular hydrogen-bonding and/or stacking interactions, may prohibit short ammine (H)···phosphate (O) distances in this solid-state structure.

The effects of platinum binding on the local structure of adjacent guanosines in single-stranded oligonucleotides may be summarized as follows. The guanine base planes are completely destacked and are locked into a structure having guanine/guanine dihedral angles of 76–87°. A switch of the 5′-sugar pucker from C2′- to C3′-endo occurs to accommodate this geometry. Although some conformational flexibility about the Pt–N7 bond and about the glycosyl bond, χ, is evident, the 17-membered ring formed by the DNA backbone restricts the possible structures to some degree. In X-ray studies of different salts of *cis*-[$Pt(NH_3)_2(9\text{-EtG})_2]^{2+}$, the only model complex of nucleobases found to have head-to-head, rather than head-to-tail, base orientations to date, the base/base dihedral angles were somewhat smaller (68–78°).[13] It will be interesting to learn whether the large guanine/guanine dihedral angles found in X-ray structures of bifunctional platinum–DNA adducts thus far will also occur in structures of platinum bound in this manner to duplex oligonucleotides.

Relatively little experimental information is available about the conformational changes that occur in nucleotides that flank the *cis*-[$Pt(NH_3)_2${d(pGpG)}] intrastrand adduct. NMR spectra of *cis*-[$Pt(NH_3)_2${d(CpGpG)}] and *cis*-[$Pt(NH_3)_2${d(ApTpGpG)}] exhibit some temperature dependence, especially for resonances of the internal guanosines and their adjacent 5′-nucleosides (C or T),[184,188] suggestive of stacking at lower temperatures. In these and other platinated oligonucleotides containing a 5′-nucleoside adjacent to the modified guanosines, a less pronounced downfield shift of the 5′-guanosine H8 resonance, relative to the 3′-guanosine H8 resonance, is observed. For *cis*-[$Pt(NH_3)_2${d(CpGpG)}], this result has been attributed to stacking between C(1) and G(2).[184] The X-ray structure of *cis*-[$Pt(NH_3)_2${d(CpGpG)}] reveals no additional information about this interaction.[190] In the crystalline state, orientation of the free 5′-cytosine base is governed by crystal-packing forces and does not necessarily reflect the conformation in solution. The NMR spectra of platinated oligonucleotides other than *cis*-[$Pt(NH_3)_2${d(CpGpG)}] and *cis*-[$Pt(NH_3)_2${d(ApTpGpG)}] demonstrate little temperature dependence in solution.[172,191]

Interactions with the 5′-nucleotide adjacent to the d(GpG) platinum adduct may modify the structure of the phosphodiester unit linking the two platinated guanines. In *cis*-[$Pt(NH_3)_2${d(GpG)}], the ^{31}P NMR spectrum is characteristic of a structure in which normal, gauche/gauche conformations occur for the phosphate ester torsion angles α and ζ.[183] In *cis*-[$Pt(NH_3)_2${d(CpGpG)}][184] and all longer single- and double-stranded oligonucleotides studied,[162,191] however, and in natural and synthetic DNAs,[173,176,177] an approximately 1.0–1.6 ppm downfield shift of the interguanosine ^{31}P resonance is observed when *cis*-$\{Pt(NH_3)_2\}^{2+}$ binds to N7 atoms of two adjacent guanosines. The origins of this effect are not understood. Such a downfield shift is usually attributed to conversion of either of the two torsion angles, α or ζ, to the trans conformation.[198,199] A decrease in the phosphodiester O–P–O bond angle may also be responsible.[198] A trans conformation about the interguanosine phosphodiester bond, α, was observed for one disordered nucleotide in the X-ray structure of *cis*-[$Pt(NH_3)_2${d(pGpG)}].[192] Molecule 1 in the X-ray structure of *cis*-[$Pt(NH_3)_2${d(GpGpG)}] also exhibits a slightly unusual value for torsion angle α,[190] but this distortion may not be statistically significant (vide supra).

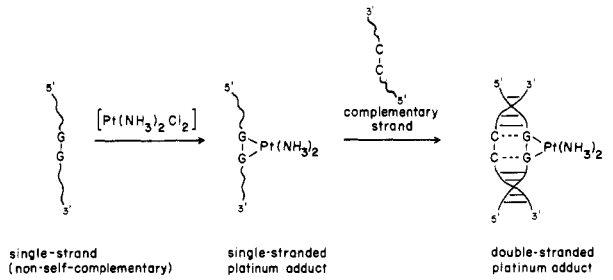


Figure 19. Strategy for synthesizing a double-stranded platinated oligonucleotide. In the first step, a non-self-complementary single-stranded oligonucleotide is allowed to react with *cis*- or *trans*-DDP. The resulting single-stranded platinum adduct is purified. Subsequently, the complementary strand is added to produce the double-stranded platinum adduct.

2. Double-Stranded Adducts

Three double-stranded oligonucleotides containing *cis*-$\{Pt(NH_3)_2\}^{2+}$ coordinated at N7 positions of adjacent guanosines have been studied by NMR spectroscopy (Table III).[162,170–172] These molecules were synthesized by adding an equimolar amount of a platinated, non-self-complementary single-stranded oligonucleotide to its complementary strand (Figure 19). In one of these single-stranded oligonucleotides, d(GpApTpCpCpG*pG*pC),[171] the modified guanosines occur near the 3′-end of the sequence, while in the others, d(TpCpTpCpG*pG*pTpCpTpC)[162,170] and d(GpCpCpG*pG*pApTpCpGpC),[172] the modified guanosines occur in the middle of the sequence. The asterisks here and elsewhere denote the sites of platinum binding.

Binding of platinum substantially destabilizes the duplex sequences d(GpApTpCpCpG*pG*pC) and d(TpCpTpCpG*pG*pTpCpTpC). The respective melting temperatures are lowered upon platination from 55 to 28 °C for the former and from 29 to 14 °C for the latter.[170,171] In the G-C-rich sequence, d(GpCpCpG*pG*pApTpCpGpC), binding of platinum lowers the duplex melting temperature from 58 to 49 °C.[172]

Further details of the melting behavior of each of the platinated complexes were obtained by observing the imino resonances (guanine N1-H and thymine N3-H), involved in Watson–Crick base pairing, as a function

of temperature. As these protons begin to exchange with H_2O, their signals broaden and eventually disappear.[200] In unmodified double-stranded oligonucleotides, melting starts at the 5'- and 3'-termini and eventually reaches the middle of the duplex. Thus, imino resonances associated with base pairs at the two ends begin to broaden first. This behavior was observed for all three of the unmodified duplexes.[170-172] For the platinated duplexes, changes both in the chemical shifts of the imino resonances and in their melting behavior were observed. Surprisingly, however, in all three sequences, the same number of imino resonances appeared in the spectra of platinated oligonucleotides at low temperature as in those of the unmodified oligonucleotides.[170-172]

The effects of coordinated *cis*-$\{Pt(NH_3)_2\}^{2+}$ on the imino resonances of duplex oligonucleotides are illustrated by the sequence d(TpCpTpCpGpGpTpCpTpC) containing a platinum cross-link at G(5) and G(6), annealed to its complementary strand (Figure 20). NOE experiments and ^1H resonance melting behavior were used to assign imino resonances to respective base pairs in both the unmodified and platinated oligonucleotide duplexes.[170] Upon platination, four imino resonances belonging to the four base pairs at the center of the duplex shift substantially. Three resonances, assigned to base pairs involving modified G(5) and G(6) and unmodified T(7), shift downfield. The imino resonance of the base pair involving C(4) shifts to higher field. Similar behavior was observed for the platinated sequence d(GpApTpGpCpG*pG*pC).[171] Thus, coordination of platinum appears to affect mainly these four base pairs. Comparison of the melting behavior of modified and unmodified oligonucleotides (Figure 20) reveals that the platinated G(5) imino proton resonance begins to exchange with water at lower temperatures than other imino resonances. The imino resonance belonging to the G(6)-containing base pair behaves normally.[162] In the case of double-stranded, platinated d(GpCpCpG*pG*pApTpCpGpC), the signal belonging to the modified base pair at the 5'-end is broadened even at low temperature.[172] At 36 °C, the imino resonances of both modified base pairs in this sequence disappear, while all of the unmodified base pairs remain intact.

In discussing the implications of these results, it must be emphasized that only the imino resonances were studied. No information was provided about the amino hydrogen bonds, of which there are two in normal, Watson–Crick G-C base pairs and one in A-T base pairs. Only where both imino and amino hydrogen bonds are observed can intact Watson–Crick base pairing be claimed. Moreover, there is no proof that the imino resonances observed at low temperature in the platinated oligonucleotides participate in the same sort of hydrogen bonding as exists under normal conditions. The theoretical models derived from molecular mechanics calculations described in the following section have suggested that unusual base pairing can occur in platinated oligonucleotides,[163-165] which is not inconsistent with these NMR results.

The structure of the platinated oligonucleotide duplex d(TpCpTpCpG*pG*pTpCpTpC) has been more extensively studied by two-dimensional NMR techniques.[162] The observed NOE's of the modified residues

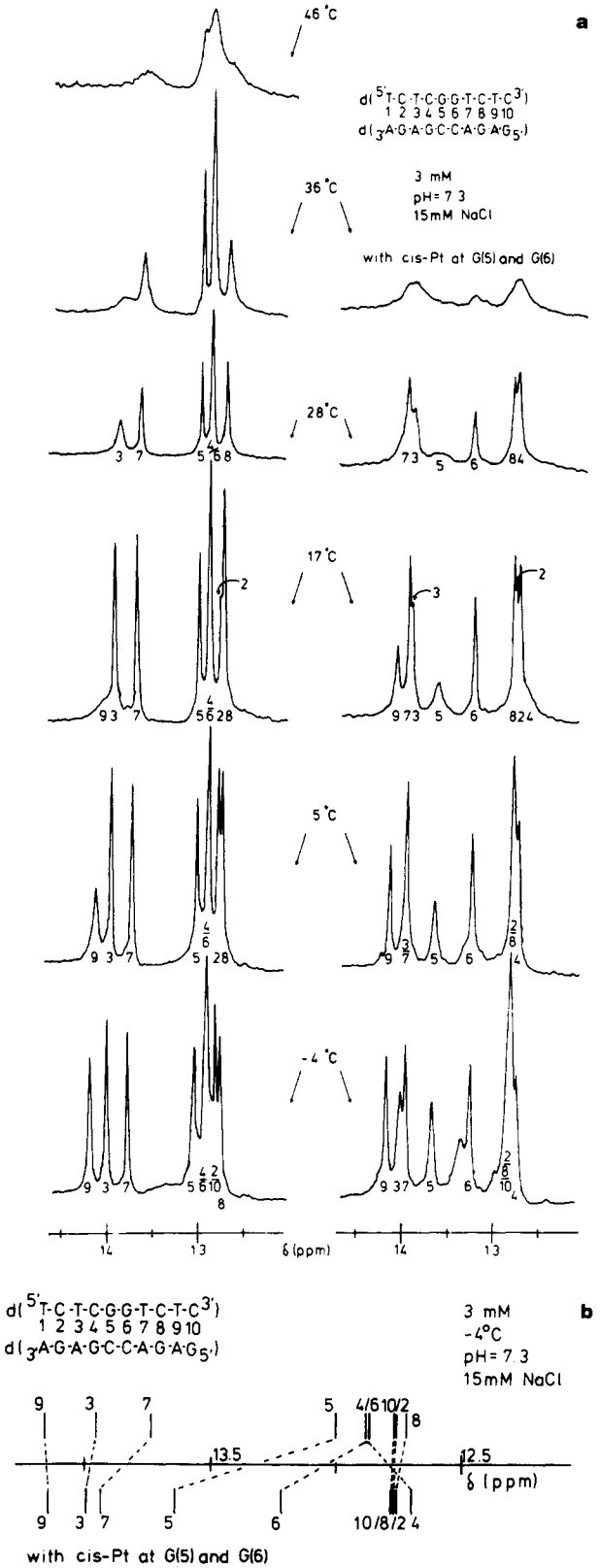


Figure 20. (a) Low-field region of the 500-MHz ^1H NMR spectra of unmodified (left) and platinated (right) duplex d-(TpCpTpCpGpGpTpCpTpC) (3 mM decamer, 93% H_2O/7% D_2O, pH 7.3, 15 mM NaCl). Chemical shifts are reported relative to DSS. The shorthand base-pair notation depicted in the right upper corner is used. The unassigned peak at 13.4 ppm in the −4 °C spectrum of the platinated oligonucleotide originates probably from base pair T·A (1–20). (b) Schematic representation of chemical shift changes of imino protons upon platinum binding: top trace, unmodified; bottom trace, platinated oligonucleotide. Chemical shifts were measured at −4 °C. Reproduced with permission from ref 170. Copyright 1984 American Chemical Society.

in the double-stranded oligonucleotide resemble those in analogous, single-stranded adducts. Thus, the gross structures of *cis*-[$Pt(NH_3)_2${d(GpG)}] adducts in both single- and double-stranded oligonucleotides appear to be similar: i.e., both guanosines are in a head-to-head, anti conformation, the 5′-sugar adopts an N-type conformation, and the 3′-sugar adopts an S-type conformation. An NOE between guanine H8 resonances has also been observed in the platinated heterocopolymer poly(dI)·poly(dC), suggesting that a head-to-head structure also occurs in higher molecular weight DNA.[173] Upon platination of the double-stranded decamer, only nonexchangeable protons belonging to residues in the central four base pairs display substantial chemical shift changes (>0.1 ppm). Specifically, most of the protons of G(5) are deshielded, while protons of G(6) are shielded. Again, it appears that only local structure is affected by coordination of *cis*-$\{Pt(NH_3)_2\}^{2+}$. The four base pairs at both ends of the molecule appear to retain normal, B-DNA-like structure.

Because the modified decanucleotide exhibits both shielding and deshielding effects relative to the unmodified duplex, the authors propose a kinked structure, in which coordination by *cis*-$\{Pt(NH_3)_2\}^{2+}$ and concomitant destacking of the modified guanosines create a bend in the DNA helix, possibly without base-pair disruption.[162] As described previously, some unpairing of bases occurs with increasing temperature (Figure 20). Both effects may combine at biological temperature to accommodate the constraints imposed by platinum binding.

3. Single- and Double-Stranded Models

Molecular mechanics calculations have been carried out on adducts of *cis*-$\{Pt(NH_3)_2\}^{2+}$ with [d(GpGpCpCpG*pG*pCpC)-d(GpGpCpCpGpGpCpC)] and [d(TpCpTpCpG*pG*pTpCpTpC)-d(GpApGpApCpCpGpApGpA)].[163-165] From these calculations, three duplex models, two of which display a kink in the helix axis, have been derived (Figure 21). Similar calculations have also been carried out on platinum bound to single-stranded oligonucleotides, thus permitting one to test their predictive value.

Indeed, the features of models of the single-stranded d(GpG) platinum adducts are similar to those observed experimentally. As in the X-ray structures of *cis*-[$Pt(NH_3)_2${d(pGpG)}] and *cis*-[$Pt(NH_3)_2${d(CpGpG)}], the interplanar angle between guanosine base planes is 75 ± 4° in these models. Moreover, the sugar conformation of the 5′-guanosine in the theoretical models switches to an N (C3′-endo) conformation, as found experimentally. In addition, both experimentally observed ammine···phosphate and ammine···guanine (O6) intramolecular hydrogen bonds are present in the theoretical models. The former, having H···O distances of 1.76–1.79 Å, is stronger than the latter, which is characterized by H···O distances of 2.04–2.27 Å.

All three duplex models retain C3′-endo conformations for the 5′-guanosine, although the base/base dihedral angles are smaller (60 ± 2° for the unkinked model and 63 ± 3° for the kinked models) than in the single-stranded models. Moreover, the ammine···phosphate hydrogen bond is intact in all of the duplex models, demonstrating that, even when the conformation of the phosphodiester link 5′ to the coordinated platinum is constrained, such a bond may form. Diminished N7–Pt–N7 angles of 76–82° were observed in all of the duplex models.

In the unkinked model, the platinated 5′-guanosine tilts off the base stack, resulting in large interplanar angles to both its 5′- and 3′-neighbors. Tilting of this residue disrupts its normal Watson–Crick base pairing, resulting in the formation of a bifurcated hydrogen bond between the two strands. Watson–Crick hydrogen bonding between O6 of guanosine and H4 of the opposite cytosine is maintained, but the hydrogen bond involving the H2-amino group is completely disrupted.

The other two platinated duplex models are kinked at angles of 61 and 49°, respectively, toward the major groove. Kinking of the helix axis at the two modified guanosines allows nearly normal stacking interactions between each coordinated guanine base and its neighbor. In the kinked models, an intramolecular hydrogen bond occurs between the 3′-guanosine O6 atom and an ammine ligand, in addition to the ammine···phosphate hydrogen bond involving the 5′-nucleoside. In the so-called "low-salt" kinked model, all of the Watson–Crick base pairs are intact, while in the "high-salt" model only one hydrogen bond occurs between the 5′-guanosine and the complementary cytosine on the opposite strand.

It must be emphasized that these theoretical models only suggest, and do not prove, how binding of platinum might affect the structures of oligonucleotides. It is impossible to conclude, based only on calculations where solvent molecules and hydrated counterions are omitted from consideration, whether the intramolecular ammine···phosphate hydrogen bond or the weaker intramolecular ammine···O6 hydrogen bond is important in stabilizing the intrastrand d(GpG)–platinum adduct in DNA. Nevertheless, the calculations are valuable in revealing nonbonded steric repulsions that limit the kinds of structures that can be imagined and in identifying possibilities for hydrogen-bonding interactions.

D. Intrastrand Binding of *cis*-$\{Pt(NH_3)_2\}^{2+}$ at the N7 Atoms of (ApG)-Containing Oligonucleotides

The dinucleoside monophosphate (ApG) reacts with *cis*-DDP in a two-step reaction to produce one major adduct in better than 95% yield. The product contains platinum chelated through the N7 atoms of adenosine and guanosine (Table III).[128] Information derived from NMR studies of this adduct suggests that its structure is not unlike that of previously described *cis*-[$Pt(NH_3)_2${d(GpG)}]. Specifically, the nucleosides display anti configurations, and the sugar conformations of adenosine and guanosine are C3′-endo and C2′-endo, respectively.[128] Studies of the complex *cis*-[$Pt(NH_3)_2$-{d(GpA*pG*)}] also revealed a C3′-endo sugar pucker for one of the residues.[129]

Recent experiments carried out with monoclonal antibodies indicate that the structure formed when *cis*-DDP binds to d(ApG) sequences in DNA is immunochemically very similar to that of the platinated d(GpG) adduct.[201] These monoclonal antibodies, which are specific for the *cis*-[$Pt(NH_3)_2${d(GpG)}] adduct on DNA, recognize conformational changes in the DNA double helix rather than the platinum adduct itself. Binding of antibodies to *cis*-DDP–DNA was competitively inhibited in an ELISA by *cis*-DDP–poly[d(AG)]·poly[d-

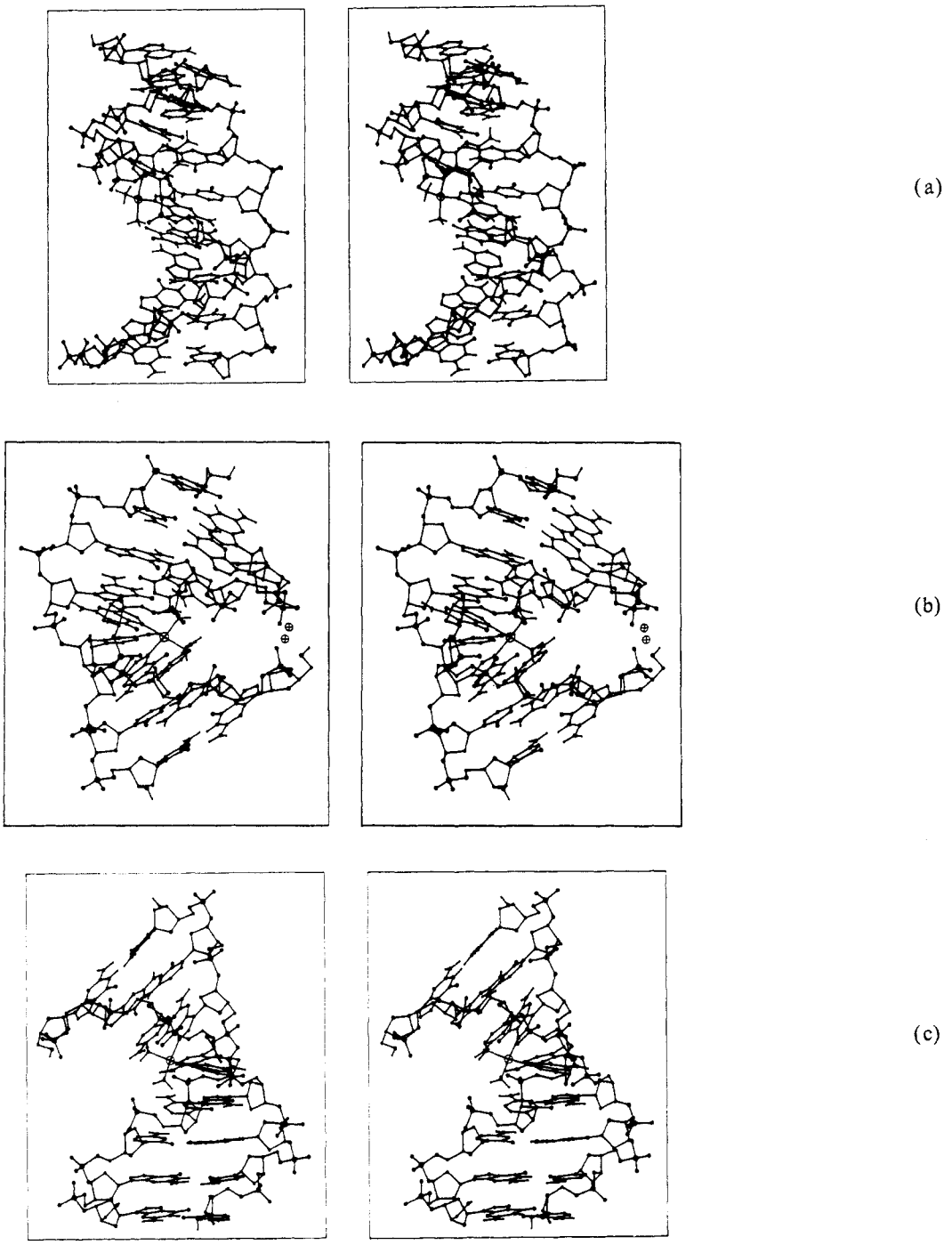


Figure 21. (Top) Stereoscopic view of the unkinked, platinated model of duplex d(TpCpTpCpG*pG*pTpCpTpC) from molecular mechanics calculations. Counterions used to stabilize the negative charge of the phosphates are not shown. (Center) Stereoscopic view of the "high-salt" kinked, platinated model of duplex d(GpGpCpCpG*pG*pCpC) from molecular mechanics calculations. Counterions are not depicted, with the exception of the bridging ion. (Bottom) Stereoscopic view of the "low-salt" kinked, platinated model of duplex d(GpGpCpCpG*pG*pCpC). Counterions are not depicted. Reproduced with permission from ref 165. Copyright 1987 Wiley.

(TC)] (D/N = 0.036), although higher concentrations of this modified synthetic polymer than of *cis*-DDP–poly(dG)·poly(dC) (D/N = 0.032) were required for equivalent inhibition of antibody binding.

E. Intrastrand *cis*-Diammineplatinum(II) Cross-Links of Two Guanosines Separated by an Intervening Nucleotide

When *cis*-DDP is allowed to react with oligonucleotide sequences containing only two guanosines separated by an intervening nucleotide (Table III), the main reaction product is a 1,3-intrastrand cross-link between the guanosine N7 atoms.[129,186,202-205] This adduct accounts for approximately 90% of the total optical density of the resulting mixture when *cis*-DDP reacts with d(GpCpG).[205] Although the self-complementary oligonucleotides d(pCpGpCpG), d(pGpCpGpC), and d(CpGpCpG) were allowed to react with *cis*-DDP at a temperature where a large proportion are double-stranded, the only products formed are intrastrand cross-links.[186] No higher molecular weight products, indicative of interstrand cross-linking, were observed.

The nonexchangeable deoxyribose and base protons of d(GpCpG) and its platinum adduct were assigned by

decoupling and NOE experiments.[202] Using spectral simulation methods, chemical shift data and coupling constants were obtained. Compared to the free oligonucleotide, which exhibits a normal temperature-dependent helix ⇌ coil transition, the chemical shifts of *cis*-[$Pt(NH_3)_2${d(GpCpG)}] are virtually temperature independent. Comparison of the NMR spectra of d(GpCpG) and its platinated product also revealed pronounced downfield shifts of all protons located on the central -pCp- residue of the adduct. Moreover, when compared to the NMR spectra of d(pCp) and the corresponding mononucleotide complexes *cis*-[$Pt(NH_3)_2${d(Gp)}$_2$] and *cis*-[$Pt(NH_3)_2${d(pG)}$_2$], none of the protons of *cis*-[$Pt(NH_3)_2${d(GpCpG)}] displayed substantial relative shieldings normally indicative of intrastrand base–base stacking interactions. A small amount of relative shielding (0.2 ppm) is exhibited by the H8 nuclei of *cis*-[$Pt(NH_3)_2${d(GpCpG)}]. Chelation of the guanosine N7 atoms of d(GpNpG) by platinum constrains them to be approximately 2.8 Å apart. The resulting proximity of the two guanosines contributes to the relative shieldings displayed by the guanosine H8 nuclei. On the other hand, the NMR data indicate that the remaining protons are relatively deshielded, especially those of the cytidine residue, compared to their chemical shift values in a stacked, helical structure. It was concluded that cytidine is destacked, or turned away, from the terminal purines in a "bulged-out" structure.[202]

Further conformational details indicate this "bulged-out" structure to be quite flexible, independent of temperature. NOE experiments yielded information about the glycosyl torsion angles of *cis*-[$Pt(NH_3)_2${d(GpCpG)}].[202] Irradiation of the H6 resonance of cytosine resulted in an NOE at its H2′ position, characteristic of an anti conformation (Figure 6). Upon irradiation at either guanosine H8 resonance, NOE's were observed at both the H1′ and H2′ signals. Therefore, both guanosines are probably undergoing rapid syn ⇌ anti equilibria on the NMR time scale or, possibly, are locked into a conformation somewhere between syn and anti extremes. From the coupling constants, the conformational populations (N or S) of the sugars were derived.[202] The results are G(1) = 59% S, C(2) = 82% S, and G(3) = 56% S at 23 °C. At higher temperatures, the populations are almost identical. Thus, the guanosine sugars exhibit substantial conformational motion relative to the cytidine sugar. Backbone angles β and γ also display a significant, temperature-independent conformational flexibility, further indicating a nonhelical structure of the molecule.[202]

Addition of bases 5′ or 3′ to the d(GNG) adduct, as in platinum complexes of d(CpG*pCpG*), d(pCpG*pCpG*), d(pG*pCpG*pC),[186] and d(TpCpTpCpG*pTpG*pTpCpTpC),[204] does not result in appreciable chemical shift changes for either of the guanosine H8 resonances. An NOE experiment performed on the guanosine H8 resonances of the single-stranded adduct *cis*-[$Pt(NH_3)_2${d(TpCpTpCpG*pTpG*pTpCpTpC)}][204] however, indicated an anti conformation for one of the bases and a syn conformation for the other. The H8 resonances were not assigned to their respective bases. These results are in conflict with those previously obtained for *cis*-[$Pt(NH_3)_2${d(GpCpG)}], where syn ⇌ anti equilibria, or a locked conformation between syn and anti (vide supra), was observed for the coordinated guanosines. Additional bases at both ends of this platinum adduct clearly influence its conformation.

After addition of the complementary strand to *cis*-[$Pt(NH_3)_2${d(TpCpTpCpG*pTpG*pTpCpTpC)}], and to the corresponding unmodified undecamer, imino protons in the low-field portion of the NMR spectra (12–15 ppm) were studied at various temperatures.[204] For the unmodified double-stranded undecamer, the imino resonances corresponding to base pairs at the ends of the molecule began to disappear as the temperature was raised, indicative of fraying. Between 53 and 58 °C, the imino protons corresponding to base pairs at the center of the sequence also broadened and finally disappeared. In the case of the platinated double-stranded oligonucleotide, an H3 proton of a thymine base not involved in hydrogen bonding was observed at 11.23 ppm at low temperature (-1 °C) and pH 5.2. This resonance was attributed to the thymidine base located between the two coordinated guanosines and is thought to be "bulged-out" from the stacked array of nucleobases, as in similar single-stranded adducts. No NOE's were observed between this H3 resonance and protons of neighboring bases, further supporting the "bulged-out" structure. Integration of the guanosine H1 imino resonances indicated that one other base pair is melted out, even at low temperature. Some evidence suggested that this base pair involved the 3′-platinated guanosine.[204] Binding of platinum at the d(GTG) site lowers the melting temperature of the undecamer from 39 to 13 °C in 0.1 M NaCl with added trace Mg^{2+} ion.[204] These results indicate that distortions caused by intrastrand binding of *cis*-$\{Pt(NH_3)_2\}^{2+}$ to two guanosines separated by an intervening nucleotide are more severe than those caused by coordination of adjacent guanosines.

F. NMR Studies of DNA Adducts of *trans*-DDP

As with *cis*-DDP, *trans*-DDP can coordinate through the N7 atoms of two guanosine residues separated by an intervening nucleotide. In the resulting adduct, however, the distance between the two N7 atoms is expected to be approximately 4.0 Å, compared to 2.8 Å for *cis*-DDP. Therefore, one might expect the two structures to be somewhat different. The structures of such *trans*-$\{Pt(NH_3)_2\}^{2+}$–oligonucleotide adducts have just begun to be studied (Table III).[203,206,207]

Reactions of *trans*-DDP with d(GpCpG) and [d(ApGpGpCpCpT)]$_2$ were monitored by HPLC methods. During the courses of both reactions, two intermediates were observed, which subsequently converted to one major single-stranded product having one platinum bound per strand. This adduct was purified and identified as the 1,3-intrastrand cross-link.[206,207] For d(ApGpGpCpCpT)]$_2$, this kind of adduct is achieved by binding of platinum to N7 atoms of A(1) and G(3).[207] Thus, reactions of *trans*-DDP with oligonucleotides occur in a two-step process, similar to those of *cis*-DDP.[172] In the reaction with d(GpCpG), the 1,3-intrastrand adduct accounted for ~70% of the total UV-absorbing material, while unreacted starting material accounted for 21%.[206] Under the conditions of its reaction with *trans*-DDP, unmodified [d(ApGpGpCpCpT)]$_2$ is double-stranded.[191] In addition

TABLE IX. Comparison of the Proton Chemical Shifts of d(GpCpG), *cis*-[Pt(NH$_3$)$_2${d(GpCpG)}], and *trans*-[Pt(NH$_3$)$_2${d(GpCpG)}]^a (Adapted from Ref 206)

species	nucleotide	H1′	H3′	H8	H5	H6	ref
d(GpCpG)	Gp–	6.136	4.848	7.865			
	–pCp–	6.123	4.789		5.788	7.554	202
	–pG	6.190	4.714	7.978			
cis-[Pt(NH$_3$)$_2${d(GpCpG)}]	Gp–	6.222	4.861	8.216			
	–pCp–	6.322	4.823		6.024	7.820	202
	–pG	6.244	4.598	8.320			
trans-[Pt(NH$_3$)$_2${d(GpCpG)}]	Gp–	6.407	4.598	8.586			
	–pCp	6.477	5.177		5.850	7.985	206
	–pG	6.311	4.768	8.713			

^{a} Chemical shifts are given (ppm) vs. Me$_4$Si.

to single-stranded products, several polymeric species, proposed to contain interstrand cross-links, were observed.[207] High molecular weight products were also seen in the reaction of *trans*-DDP with d(GpTpG), in addition to the major adduct.[203] The relative variety of adducts produced in these reactions of oligonucleotides with *trans*-DDP, compared to similar reactions with *cis*-DDP, indicates a less pronounced regiospecificity for binding, as noted previously.[70]

For *trans*-[Pt(NH$_3$)$_2${d(GpTpG)}][203] and *trans*-[Pt(NH$_3$)$_2${d(GpCpG)}],[206] a larger downfield shift of the guanosine H8 resonances, compared to the corresponding cis complexes, was observed. This effect has also been noted in bis(nucleotide) complexes of *cis*- and *trans*-DDP[208] and can be attributed to the relative lack of mutual shielding by two trans-oriented bases.

With the aid of two-dimensional COSY and NOE experiments, most of the nonexchangeable proton resonances were assigned for the complexes *trans*-[Pt(NH$_3$)$_2${d(ApGpGpCpCpT)}][207] and *trans*-[Pt(NH$_3$)$_2${d(GpCpG)}].[206] Since the resonances in the proton spectrum of the *cis*-{Pt(NH$_3$)$_2$}$^{2+}$ adduct of d(GpCpG) had been previously assigned, a direct comparison was possible (Table IX).[206] Almost all of the protons of the trans adduct of d(GpCpG) resonate at lower fields than those of *cis*-[Pt(NH$_3$)$_2${d(GpCpG)}]. The relative downfield shifts are especially obvious for protons of the cytidine residue. Although the exact chemical shifts of the H2′ resonances in the NMR spectrum of *trans*-[Pt(NH$_3$)$_2${d(GpCpG)}] could not be assigned owing to spectral overlap, the H2′ resonance of the –pCp– residue is strongly deshielded in comparison with both *cis*-[Pt(NH$_3$)$_2${d(GpCpG)}] and d(GpCpG). As in the analysis of *cis*-[Pt(NH$_3$)$_2${d(GpCpG)}], the relative deshieldings of *trans*-[Pt(NH$_3$)$_2${d(GpCpG)}] compared to unmodified d(GpCpG) demonstrate destacking of the –pCp– residue. Inspection of the values in Table IX indicates that this destacking effect may be even more pronounced than that which occurs in the cis adduct.

Several features shared by the *trans*-{Pt(NH$_3$)$_2$}$^{2+}$ complexes of d(GpCpG)[206] and d(ApGpGpCpCpT)[207] reveal the structures of the A(1),G(3) and G(1),G(3) intrastrand cross-links to be similar. In *trans*-[Pt(NH$_3$)$_2${d(ApGpGpCpCpT)}], less shielding of the proton resonances of G(2) are observed compared to unmodified d(ApGpCpCpCpT). Moreover, the G(2) H8 resonance is readily broadened by paramagnetic trace metal ions, demonstrating its accessibility to bulk solvent.[207] Therefore, similar structures occur for the two trans-1,3-adducts, where the intervening nucleoside base is destacked and probably turned away from the flanking bases. For both adducts,[206,207] an N-type

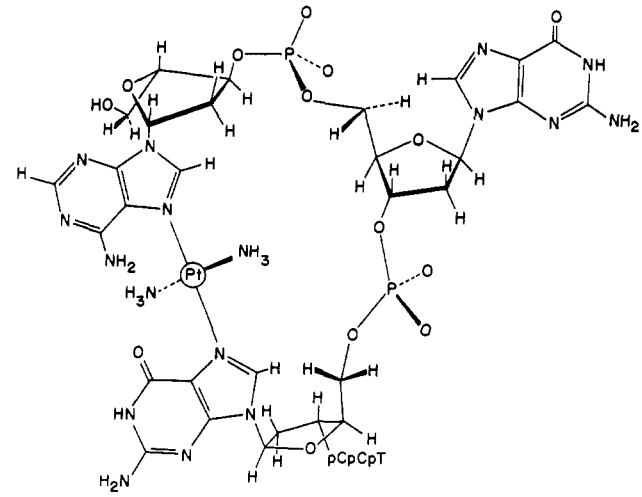


Figure 22. Schematic illustration of *trans*-[Pt(NH$_3$)$_2${d(A*pGpG*pCpCpT)}]. See ref 207.

(C3′-endo) sugar pucker was observed for the 5′-platinated purine nucleoside. A similar N-type sugar pucker was observed in the spectrum of *trans*-[Pt(NH$_3$)$_2${d(GpTpG)}] but was not assigned.[203] A strong NOE between H3′ and H8 of A(1) was observed in the structure of *trans*-[Pt(NH$_3$)$_2${d(ApGpGpCpCpT)}],[207] consistent with predominantly anti orientation of the base. An unusual geometry for torsion angle ϵ of this residue was also noted, owing to a weak coupling between H3′ and P. These results reveal that *trans*-{Pt(NH$_3$)$_2$}$^{2+}$ 1,3-intrastrand adducts substantially disrupt the structure of DNA. A schematic illustration of this interaction is displayed in Figure 22.

VI. Conclusions and Future Challenges

Although major advances have been made toward understanding the mechanism of action of *cis*-DDP, many aspects of the problem remain to be sorted out. Solutions to these remaining puzzles could eventually lead to better anticancer drugs and a greater understanding of the disease itself.

We now have much evidence that DNA is the main target of *cis*-DDP. A possibility not touched upon here is that platinum binding to other cellular components may also contribute to its anticancer potency. The formation of platinum-induced DNA–protein cross-links is a related topic worthy of further investigation. The question of why *cis*-[Pt(NH$_3$)$_2$Cl$_2$] inhibits DNA replication at much lower doses than *trans*-DDP remains to be answered. Although differential repair of adducts formed by the two isomers is an attractive explanation, more work in this area is required before

definitive conclusions can be drawn.

Sequence-specific adducts formed between *cis*-DDP and DNA have now been identified and quantitated, both in vitro and in vivo. How these *cis*-DDP binding sites might be modulated by other drugs used in combination chemotherapy has just begun to be explored. The overriding question of the relative toxicities, repair, and antitumor activities of these specific adducts remains unanswered. Moreover, relationships between mutagenicity and toxicity of specific adducts have not yet been established. Genetically engineered plasmids containing site-specific adducts, such as that described in section IVD, will be invaluable in responding to these challenges, as will be antibodies raised to probe various adducts in vivo.

Valuable information can also be gained from comparable studies of adducts formed between *trans*-DDP and DNA, since the latter is inactive and inhibits DNA replication within cells much less efficiently than *cis*-DDP at equivalent doses of the two isomers. What are the kinetics of reactions between *trans*-DDP and DNA in tumor cells? Are long-lived monofunctional adducts produced by *trans*-DDP, and are they the reason why this isomer is biologically inactive? What kinds of bifunctional adducts does *trans*-DDP produce? New experimental methods may need to be developed in order to address these questions.

Further structural studies of platinum binding modes will be important for understanding, at the molecular level, why certain adducts result in anticancer activity while others do not. Although the structure of the single-stranded, platinum-induced cross-link of adjacent guanosine N7 atoms has now been established, structural details of other single-stranded adducts formed by both *cis*- and *trans*-DDP still need to be deduced. More importantly, structures of double-stranded oligonucleotide adducts have yet to be elucidated. To what extent does binding of platinum disrupt Watson–Crick base pairing in duplex DNA? What is the structure of the interstrand cross-link? These are just some examples of questions that can be addressed by NMR and X-ray studies on platinated deoxyoligonucleotide duplexes.

VII. Acknowledgment

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VIII. References

(1) Werner, A. *Z. Anorg. Chem.* **1893**, *3*, 267.
(2) Hacker, M. P., Douple, E. B., Krakoff, I. H., Eds. *Platinum Coordination Complexes in Cancer Chemotherapy*; Martinus Nijhoff: Boston, 1984.
(3) Reedijk, J.; Lohman, P. H. M. *Pharm. Week. Sci. Ed.* **1985**, *7*, 173.
(4) Prestayko, A. W., Crooke, S. T., Carter, S. K., Eds. *Cisplatin: Current Status and New Developments*; Academic: New York, 1980.
(5) Loehrer, P. J.; Einhorn, L. H. *Ann. Intern. Med.* **1984**, *100*, 704.
(6) Roberts, J. J.; Thomson, A. J. *Prog. Nucl. Acids Res. Mol. Biol.* **1979**, *22*, 71.
(7) Roberts, J. J.; Pera, M. F., Jr. In *Platinum, Gold, and Other Metal Chemotherapeutic Agents*; Lippard, S. J., Ed.; ACS Symposium Series 209, American Chemical Society: Washington, DC, 1983; p 3.
(8) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
(9) IUPAC-IUB Joint Commission on Biochemical Nomenclature Report *Eur. J. Biochem.* **1983**, *131*, 9.
(10) Pinto, A. L.; Lippard, S. J. *Biochim. Biophys. Acta* **1985**, *780*, 167.
(11) Kistenmacher, T. J.; Orbell, J. D.; Marzilli, L. G. In *Platinum, Gold, and Other Metal Chemotherapeutic Agents*; Lippard, S. J., Ed.; ACS Symposium Series 209; American Chemical Society: Washington, DC, 1983; p 191.
(12) Marcelis, A. T. M.; Reedijk, J. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 121.
(13) Schöllhorn, H.; Raudaschl-Sieber, G.; Müller, G.; Thewalt, U.; Lippert, B. *J. Am. Chem. Soc.* **1985**, *107*, 5932 and references cited therein.
(14) Martin, R. B. In *Platinum, Gold, and Other Metal Chemotherapeutic Agents*; Lippard, S. J., Ed.; ACS Symposium Series 209; American Chemical Society: Washington, DC, 1983; p 231.
(15) Rosenberg, B.; VanCamp, L.; Krigas, T. *Nature (London)* **1965**, *205*, 698.
(16) Rosenberg, B.; VanCamp, L.; Grimley, E. B.; Thomson, A. J. *J. Biol. Chem.* **1967**, *242*, 1347.
(17) Rosenberg, B.; VanCamp, L.; Trosko, J. E.; Mansour, V. H. *Nature (London)* **1969**, *222*, 385.
(18) Rosenberg, B. In *Nucleic Acid-Metal Ion Interactions*; Spiro, T. G., Ed.; Wiley: New York, 1980; p 3.
(19) Barnard, C. F. J.; Cleare, M. J.; Hydes, P. C. *Chem. Brit.* **1986**, *22*, 1001.
(20) Tullius, T. D.; Ushay, H. M.; Merkel, C. M.; Caradonna, J. P.; Lippard, S. J. In *Platinum, Gold, and Other Metal Chemotherapeutic Agents*; Lippard, S. J., Ed.; ACS Symposium Series 209; American Chemical Society: Washington, DC, 1983; p 51.
(21) Howe-Grant, M. E.; Lippard, S. J. In *Metal Ions in Biological Systems*; Sigel, H., Ed.; Marcel Dekker: New York, 1980; Vol. II, p 63.
(22) Withens, E. M. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 1275.
(23) Rosenkranz, H. S.; Garro, A. J.; Levy, J. A.; Carr, H. S. *Biochim. Biophys. Acta* **1966**, *114*, 501.
(24) Setlow, R. B.; Swenson, P. A.; Carrier, W. L. *Science (Washington, D.C.)* **1963**, *142*, 1464.
(25) Young, C. W.; Hodas, S. *Science (Washington, D.C.)* **1964**, *146*, 1172.
(26) Harder, H. C.; Rosenberg, B. *Int. J. Cancer* **1970**, *6*, 207.
(27) Howle, J. A.; Gale, G. R. *Biochem. Pharmacol.* **1970**, *19*, 2757.
(28) Harder, H. C.; Smith, R. G.; Leroy, A. F. *Cancer Res.* **1976**, *36*, 3821.
(29) Reslova, S. *Chem.-Biol. Interact.* **1971**, *4*, 66.
(30) Reslova-Vasilukova, S. In *Recent Results in Cancer Research*; Connors, T. A., Roberts, J. J., Eds.; Springer: Berlin, 1974; Vol. 48, p 105.
(31) Pasco, J. M.; Roberts, J. J. *Biochem. Pharmacol.* **1974**, *23*, 1345.
(32) Johnson, N. P.; Hoeschele, J. D.; Rahn, R. O.; O'Neill, J. P.; Hsie, A. W. *Cancer Res.* **1980**, *40*, 1463.
(33) Alazard, R.; Germanier, M.; Johnson, N. P. *Mutat. Res.* **1982**, *93*, 327.
(34) Salles, B.; Butour, J.-L.; Lesca, C.; Macquet, J.-P. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 555.
(35) Ciccarelli, R. B.; Solomon, M. J.; Varshavsky, A.; Lippard, S. J. *Biochemistry* **1985**, *24*, 7533.
(36) Roberts, J. J.; Friedlos, F. *Cancer Res.* **1987**, *47*, 31.
(37) Beck, D. J.; Brubaker, R. R. *J. Bacteriol.* **1973**, *116*, 1247.
(38) Drobník, J.; Urbánková, M.; Krekulová, A. *Mutat. Res.* **1973**, *17*, 13.
(39) Markham, B.; Brubaker, R. R. *J. Bacteriol.* **1980**, *143*, 455.
(40) Brouwer, J.; Van de Putte, P.; Fichtinger-Schepman, A. M. J.; Reedijk, J. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 7010.
(41) Beck, D. J.; Popoff, S.; Sancar, A.; Rupp, W. D. *Nucleic Acids Res.* **1985**, *13*, 7395.
(42) Fram, R. J.; Cusick, P. S.; Wilson, J. M.; Marinus, M. G. *Mol. Pharmacol.* **1985**, *28*, 51.
(43) Popoff, S. C.; Beck, D. J.; Rupp, W. D. *Mutat. Res.* **1987**, *183*, 129.
(44) Fraval, H. N. A.; Rawlings, C. J.; Roberts, J. J. *Mutat. Res.* **1978**, *51*, 121.
(45) Aprile, F.; Martin, D. S. *Inorg. Chem.* **1962**, *1*, 551.
(46) Reishus, J. W.; Martin, D. S., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 2457.
(47) Lee, K. W.; Martin, D. S., Jr. *Inorg. Chim. Acta* **1976**, *17*, 105.
(48) Lim, M. C.; Martin, R. B. *J. Inorg. Nucl. Chem.* **1976**, *38*, 1911.
(49) Faggiani, R.; Lippert, B.; Lock, C. J. L.; Rosenberg, B. *J. Am. Chem. Soc.* **1977**, *99*, 777.
(50) Faggiani, R.; Lippert, B.; Lock, C. J. L.; Rosenberg, B. *Inorg. Chem.* **1977**, *16*, 1192.
(51) Basolo, F.; Gray, H. B.; Pearson, R. G. *J. Am. Chem. Soc.* **1960**, *82*, 4200.
(52) Gray, H. B.; Olcott, R. J. *Inorg. Chem.* **1962**, *1*, 481.
(53) Johnson, N. P.; Hoeschele, J. D.; Rahn, R. O. *Chem.-Biol. Interact.* **1980**, *30*, 151.
(54) Ushay, H. M.; Tullius, T. D.; Lippard, S. J. *Biochemistry* **1981**, *20*, 3744.

(55) Barton, J. K.; Lippard, S. J. In *Nucleic Acid-Metal Ion Interactions*; Spiro, T. J., Ed.; Wiley: New York, 1980; Vol. I, p 31.
(56) Shieh, H.-S.; Berman, H. M.; Dabrow, M.; Neidle, S. *Nucleic Acids Res.* **1980**, *8*, 85.
(57) Horáček, P.; Drobník, J. *Biochim. Biophys. Acta* **1971**, *254*, 341.
(58) Howe-Grant, M.; Wu, K. C.; Bauer, W. R.; Lippard, S. J. *Biochemistry* **1976**, *15*, 4339.
(59) Butour, J.-L.; Macquet, J.-P. *Eur. J. Biochem.* **1977**, *78*, 455.
(60) Louie, S.; Bau, R. *J. Am. Chem. Soc.* **1977**, *99*, 3874.
(61) Bose, R. N.; Cornelius, R. D.; Viola, R. E. *J. Am. Chem. Soc.* **1986**, *108*, 4403.
(62) Reily, M. D.; Marzilli, L. G. *J. Am. Chem. Soc.* **1986**, *108*, 8299.
(63) Hartley, F. R. *The Chemistry of Platinum and Palladium*; Wiley: New York, 1973.
(64) Peloso, A. *Coord. Chem. Rev.* **1973**, *10*, 123.
(65) Marzilli, L. G.; Kistenmacher, T. J.; Eichhorn, G. L. In *Nucleic Acid-Metal Ion Interactions*; Spiro, T. G., Ed.; Wiley: New York, 1980; p 179.
(66) Mansy, S.; Chu, G. Y. H.; Duncan, R. E.; Tobias, R. S. *J. Am. Chem. Soc.* **1978**, *100*, 607.
(67) Bonnaccorsi, R.; Pullman, A.; Scrocco, E.; Tomasi, J. *Theor. Chim. Acta* **1972**, *24*, 51.
(68) Bonnaccorsi, R.; Scrocco, E.; Tomasi, J.; Pullman, A. *Theor. Chim. Acta* **1975**, *36*, 339.
(69) Basch, H.; Krauss, M.; Stevens, W. J.; Cohen, D. *Inorg. Chem.* **1986**, *25*, 684.
(70) Pinto, A. L.; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4616.
(71) For example: Mansy, S.; Rosenberg, B.; Thomson, A. J. *J. Am. Chem. Soc.* **1973**, *95*, 1633.
(72) Macquet, J.-P.; Theophanides, T. *Bioinorg. Chem.* **1975**, *5*, 59.
(73) Goodgame, D. M. L.; Jeeves, I.; Phillips, F. L.; Skapski, A. C. *Biochim. Biophys. Acta* **1975**, *378*, 153.
(74) Millard, M. M.; Macquet, J.-P.; Theophanides, T. *Biochim. Biophys. Acta* **1975**, *402*, 166.
(75) Dehand, J.; Jordanov, J. *J. Chem. Soc., Chem. Commun.* **1976**, 598.
(76) Macquet, J.-P.; Theophanides, T. *Biochim. Biophys. Acta* **1976**, *442*, 142.
(77) Macquet, J.-P.; Butour, J.-L. *Biochimie* **1978**, *60*, 901.
(78) Lusty, J. R.; Chan, H. S. O.; Khor, E.; Peeling, J. *Inorg. Chim. Acta* **1985**, *106*, 209.
(79) Dijt, F. J.; Canters, G. W.; den Hartog, J. H. J.; Marcelis, A. T. M.; Reedijk, J. *J. Am. Chem. Soc.* **1984**, *106*, 3644.
(80) Raudaschl-Sieber, G.; Marzilli, L. G.; Lippert, B.; Shinozuka, K. *Inorg. Chem.* **1985**, *24*, 989.
(81) Schöllhorn, H.; Beyerle-Pfnür, R.; Thewalt, U.; Lippert, B. *J. Am. Chem. Soc.* **1986**, *108*, 3680.
(82) Roberts, J. J.; Pascoe, J. M. *Nature (London)* **1972**, *235*, 282.
(83) Zwelling, L. A.; Kohn, K. W.; Ross, W. E.; Ewig, R. A. G.; Anderson, T. *Cancer Res.* **1978**, *38*, 1762.
(84) Zwelling, L. A.; Anderson, T.; Kohn, K. W. *Cancer Res.* **1979**, *39*, 365.
(85) Zwelling, L. A.; Bradley, M. O.; Sharkey, N. A.; Anderson, T.; Kohn, K. W. *Mutat. Res.* **1979**, *67*, 271.
(86) Laurent, G.; Erickson, L. C.; Sharkey, N. A.; Kohn, K. W. *Cancer Res.* **1981**, *41*, 3347.
(87) Zwelling, L. A.; Michaels, S.; Schwartz, H.; Dobson, P. P.; Kohn, K. W. *Cancer Res.* **1981**, *41*, 640.
(88) Strandberg, M. C.; Bresnick, E.; Eastman, A. *Chem.-Biol. Interact.* **1982**, *39*, 169.
(89) Plooy, A. C. M.; Van Dijk, M.; Lohman, P. H. M. *Cancer Res.* **1984**, *44*, 2043.
(90) Murray, D.; Jenkins, W. T.; Meyn, R. E. *Cancer Res.* **1985**, *45*, 6446.
(91) Brookes, P.; Lawley, P. D. *Biochem. J.* **1961**, *80*, 496.
(92) Shooter, K. V.; Howse, R.; Merrifield, R. K.; Dobbins, A. B. *Chem.-Biol. Interact.* **1972**, *5*, 289.
(93) Munchausen, L. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4519.
(94) Plooy, A. C. M.; van Dijk, M.; Berends, F.; Lohman, P. H. M. *Cancer Res.* **1985**, *45*, 4178.
(95) Eastman, A. *Biochemistry* **1985**, *24*, 5027.
(96) Banjar, Z. M.; Hnilica, L. S.; Briggs, R. C.; Stein, J.; Stein, G. *Biochemistry* **1984**, *23*, 1921.
(97) Zwelling, L. A. In *Platinum, Gold, and Other Metal Chemotherapeutic Agents*; Lippard, S. J., Ed.; ACS Symposium Series 209; American Chemical Society: Washington, DC, 1983; p 27.
(98) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 4th ed.; Wiley: New York, 1980; p 950.
(99) Drewinko, B.; Dipasquale, M. A.; Yang, L. Y.; Barlogie, B.; Trujillo, J. M. *Chem.-Biol. Interact.* **1985**, *55*, 1.
(100) Murthy, M. S.; Travis, J. D.; Erickson, L. C.; Khandekar, J. D.; Scanlon, E. F. *Cancer Res.* **1985**, *45*, 6232.
(101) Rawlings, C. J.; Roberts, J. J. *Mutat. Res.* **1986**, *166*, 157.
(102) Sundquist, W. I.; Ahmed, K. J.; Hollis, S.; Lippard, S. J. *Inorg. Chem.* **1987**, *26*, 1524.
(103) Stone, P. J.; Kelman, A. D.; Sinex, F. M. *Nature (London)* **1974**, *251*, 736.
(104) Munchausen, L. L.; Rahn, R. O. *Cancer Chemother. Rep., Part 1* **1975**, *59*, 643.
(105) Munchausen, L. L.; Rahn, R. O. *Biochim. Biophys. Acta* **1975**, *414*, 242.
(106) Stone, P. J.; Kelman, A. D.; Sinex, F. M. *J. Mol. Biol.* **1976**, *104*, 793.
(107) Ushay, H. M.; Tullius, T. D.; Lippard, S. J. *Biochemistry* **1981**, *20*, 3744.
(108) Cohen, G. L.; Ledner, J. A.; Bauer, W. R.; Ushay, H. M.; Caravana, C.; Lippard, S. J. *J. Am. Chem. Soc.* **1980**, *102*, 2487.
(109) Tullius, T. D.; Lippard, S. J. *J. Am. Chem. Soc.* **1981**, *103*, 4620.
(110) Royer-Pokora, B.; Gordon, L. K.; Haseltine, W. A. *Nucleic Acids Res.* **1981**, *9*, 4595.
(111) Chottard, J.-C.; Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Mansuy, D. *Nouv. J. Chim.* **1978**, *2*, 551.
(112) Chottard, J.-C.; Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Mansuy, D. *J. Am. Chem. Soc.* **1980**, *102*, 5565.
(113) Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Chottard, J.-C. *Biochemistry* **1982**, *21*, 1352.
(114) Caradonna, J. P.; Lippard, S. J.; Gait, M. J.; Singh, M. *J. Am. Chem. Soc.* **1982**, *104*, 5793, and manuscript submitted for publication.
(115) Girault, J.-P.; Chottard, J.-C.; Guittet, E. R.; Lallemand, J.-Y.; Huynh-Dinh, T.; Igolen, J. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 1157.
(116) Eastman, A. *Biochemistry* **1982**, *21*, 6732.
(117) Fichtinger-Schepman, A. M. J.; Lohman, P. H. M.; Reedijk, J. *Nucleic Acids Res.* **1982**, *10*, 5345.
(118) Johnson, N. P. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 1394.
(119) Eastman, A. *Biochemistry* **1983**, *22*, 3927.
(120) Rahn, R. O. *J. Inorg. Biochem.* **1984**, *21*, 311.
(121) Fichtinger-Schepman, A. M. J.; Van der Meer, J. L.; den Hartog, J. H. J.; Lohman, P. H. M.; Reedijk, J. *Biochemistry* **1985**, *24*, 707.
(122) Johnson, N. P.; Mazard, A. M.; Escalier, J.; Macquet, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 6376.
(123) Eastman, A. *Biochemistry* **1986**, *25*, 3912.
(124) Fichtinger-Schepman, A. M. J.; van der Veer, J. L.; Lohman, P. H. M.; Reedijk, J. *J. Inorg. Biochem.* **1984**, *21*, 103.
(125) Bowler, B. E.; Lippard, S. J. *Biochemistry* **1986**, *25*, 3031.
(126) Malinge, J.-M.; Schwartz, A.; Leng, M. *Nucleic Acids Res.* **1987**, *15*, 1779.
(127) Inagaki, K.; Kidani, Y. *Inorg. Chim. Acta* **1983**, *80*, 171.
(128) van Hemelryck, B.; Girault, J.-P.; Chottard, G.; Valadon, P.; Laoui, A.; Chottard, J.-C. *Inorg. Chem.* **1987**, *26*, 787.
(129) van der Veer, J. L.; van den Elst, H.; den Hartog, J. H. J.; Fichtinger-Schepman, A. M. J.; Reedijk, J. *Inorg. Chem.* **1986**, *25*, 4657.
(130) Marcelis, A. T. M.; Erkelens, C.; Reedijk, J. *Inorg. Chim. Acta* **1984**, *91*, 129.
(131) Dewan, J. C. *J. Am. Chem. Soc.* **1984**, *106*, 7239.
(132) Reily, M. D.; Marzilli, L. G. *J. Am. Chem. Soc.* **1986**, *108*, 6785.
(133) Burnouf, D.; Daune, M.; Fuchs, R. P. P. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 3758.
(134) Marcelis, A. T. M.; van Kralingen, C. G.; Reedijk, J. *J. Inorg. Biochem.* **1980**, *13*, 213.
(135) Eapen, S. S.; Green, M.; Ismail, I. M. *J. Inorg. Biochem.* **1985**, *23*, 233.
(136) Evans, D. J.; Ford, N. R.; Green, M. *Inorg. Chim. Acta* **1986**, *125*, L39.
(137) Butour, J.-L.; Johnson, N. P. *Biochemistry* **1986**, *25*, 4534.
(138) Poirier, M. C.; Lippard, S. J.; Zwelling, L. A.; Ushay, H. M.; Kerrigan, D.; Thill, C. C.; Santella, R. M.; Grunberger, D.; Yuspa, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6443.
(139) Fichtinger-Schepman, A. M. J.; Baan, R. A.; Schutte, A.; Van Dijk, M.; Lohman, P. H. M. *Chem.-Biol. Interact.* **1985**, *55*, 275.
(140) Poirier, M. C. *J. Natl. Cancer Inst.* **1981**, *67*, 515.
(141) Plooy, A. C. M.; Fichtinger-Schepman, A. M. J.; Schutte, H. H.; Van Dijk, M.; Lohman, P. H. M. *Carcinogenesis* **1985**, *6*, 561.
(142) Poirier, M. C.; Reed, E.; Zwelling, L. A.; Ozols, R. F.; Litterst, C. L.; Yuspa, S. H. *Environ. Health Persp.* **1985**, *62*, 89.
(143) Reed, E.; Yuspa, S. H.; Zwelling, L. A.; Ozols, R. F.; Poirier, M. C. *J. Clin. Invest.* **1986**, *77*, 545.
(144) Lippard, S. J.; Ushay, H. M.; Merkel, C. M.; Poirier, M. C. *Biochemistry* **1983**, *22*, 5165.
(145) (a) Dickerson, R. E.; Drew, H. R. *J. Mol. Biol.* **1981**, *149*, 761. (b) Dickerson, R. E. *J. Mol. Biol.* **1983**, *166*, 419.

(146) Wang, A. H.-J.; Quigley, G. J.; Kolpack, F. J.; Crawford, L.; van Boom, J. H.; van der Marel, G.; Rich, A. *Nature (London)* **1979**, *282*, 680.
(147) Tullius, T. D.; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 3489.
(148) Merkel, C. M.; Lippard, S. J. *Cold Spring Harbor Symp. Quant. Biol.* **1982**, *47*, 355.
(149) Bowler, B. E.; Hollis, L. S.; Lippard, S. J. *J. Am. Chem. Soc.* **1984**, *106*, 6102.
(150) Bowler, B. E. Ph.D. Dissertation, Massachusetts Institute of Technology, 1986.
(151) Malinge, J.-M.; Leng, M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6317.
(152) Inagaki, K.; Kidani, Y. *Inorg. Chim. Acta* **1980**, *46*, 35.
(153) Harder, H. C. *Chem.-Biol. Interact.* **1975**, *10*, 27.
(154) Srivastava, R. C.; Froehlich, J.; Eichhorn, G. L. *Biochimie* **1978**, *60*, 879.
(155) Hermann, D.; Houssier, C.; Guschlbauer, W. *Biochim. Biophys. Acta* **1979**, *564*, 456.
(156) Butour, J.-L.; Macquet, J.-P. *Biochim. Biophys. Acta* **1981**, *653*, 305.
(157) Vrána, O.; Brabec, V.; Kleinwächter, V. *Anti-Cancer Drug Design* **1986**, *1*, 95.
(158) Cohen, G. L.; Bauer, W. R.; Barton, J. K.; Lippard, S. J. *Science (Washington, D.C.)* **1979**, *203*, 1014.
(159) Scovell, W. M.; Kroos, L. R. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 1597.
(160) Scovell, W. M.; Collart, F. *Nucleic Acids Res.* **1985**, *13*, 2881.
(161) Mong, S.; Daskal, Y.; Prestayko, A. W.; Crooke, S. T. *Cancer Res.* **1981**, *41*, 4020.
(162) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; van der Marel, G. A.; Haasnoot, C. A. G.; Reedijk, J. *J. Biomol. Struct. Dynam.* **1985**, *2*, 1137.
(163) Kozelka, J.; Petsko, G. A.; Lippard, S. J.; Quigley, G. J. *J. Am. Chem. Soc.* **1985**, *107*, 4079.
(164) Kozelka, J.; Petsko, G. A.; Quigley, G. J.; Lippard, S. J. *Inorg. Chem.* **1986**, *25*, 1075.
(165) Kozelka, J.; Archer, S.; Petsko, G. A.; Lippard, S. J.; Quigley, G. J. *Biopolymers* **1987**, *26*, 1245.
(166) Pinto, A. L.; Naser, L. J.; Essigmann, J. M.; Lippard, S. J. *J. Am. Chem. Soc.* **1986**, *108*, 7405.
(167) Scovell, W. M.; Capponi, V. J. *Biochem. Biophys. Res. Commun.* **1982**, *107*, 1138.
(168) Scovell, W. M.; Capponi, V. J. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 367.
(169) Houssier, C.; Macquet, M. N.; Fredericq, E. *Biochim. Biophys. Acta* **1983**, *739*, 312.
(170) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; van der Marel, G. A.; Haasnoot, C. A. G.; Reedijk, J. *J. Am. Chem. Soc.* **1984**, *106*, 1528.
(171) van Hemelryck, B.; Guittet, E.; Chottard, G.; Girault, J.-P.; Huynh-Dinh, T.; Lallemand, J.-Y.; Igolen, J.; Chottard, J.-C. *J. Am. Chem. Soc.* **1984**, *106*, 3037.
(172) van Hemelryck, B.; Guittet, E.; Chottard, G.; Girault, J.-P.; Herman, F.; Huynh-Dinh, T.; Lallemand, J.-Y.; Igolen, J.; Chottard, J.-C. *Biochem. Biophys. Res. Commun.* **1986**, *138*, 758.
(173) Reily, M. D.; Marzilli, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 4916.
(174) Sundquist, W. I.; Lippard, S. J.; Stollar, B. D. *Biochemistry* **1986**, *25*, 1520.
(175) Rubin, J. R.; Sabat, M.; Sundralingam, M. *Nucleic Acids Res.* **1983**, *11*, 6571.
(176) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; Reedijk, J. *FEBS Lett.* **1984**, *176*, 393.
(177) Marzilli, L. G.; Reily, M. D.; Heyl, B. L.; McMurray, C. T.; Wilson, W. D. *FEBS Lett.* **1984**, *176*, 389.
(178) Gassen, H. G., Lang, A., Eds. *Chemical and Enzymatic Synthesis of Gene Fragments*; Verlag Chemie: Weinheim, 1982.
(179) Gait, M. J., Ed. *Oligonucleotide Synthesis. A Practical Approach*; IRL: Oxford, 1984.
(180) Altona, C. *Recl. Trav. Chim. Pays-Bas* **1982**, *101*, 413.
(181) Kennard, O. *Biochem. Soc. Trans.* **1986**, *14*, 207.
(182) Wing, R. M.; Pjura, P.; Drew, H. R.; Dickerson, R. E. *EMBO J.* **1984**, *3*, 1201.
(183) den Hartog, J. H. J.; Altona, C.; Chottard, J.-C.; Girault, J.-P.; Lallemand, J.-Y.; de Leeuw, F. A. A. M.; Marcelis, A. T. M.; Reedijk, J. *Nucleic Acids Res.* **1982**, *10*, 4715.
(184) den Hartog, J. H. J.; Altona, C.; van der Marel, G.; Reedijk, J. *Eur. J. Biochem.* **1985**, *147*, 371.
(185) Marcelis, A. T. M.; Canters, G. W.; Reedijk, J. *Recl. Trav. Chim. Pays-Bas* **1981**, *100*, 391.
(186) Marcelis, A. T. M.; den Hartog, J. H. J.; van der Marel, G. A.; Wille, G.; Reedijk, J. *Eur. J. Biochem.* **1983**, *135*, 343.
(187) Girault, J.-P.; Chottard, J.-C.; Neumann, J.-M.; Tran-Dinh, S.; Huynh-Dinh, T.; Igolen, J. *Nouv. J. Chim.* **1984**, *8*, 7.
(188) Neumann, J.-M.; Tran-Dinh, S.; Girault, J.-P.; Chottard, J.-C.; Huynh-Dinh, T.; Igolen, J. *Eur. J. Biochem.* **1984**, *141*, 465.
(189) Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *Science (Washington, D.C.)* **1985**, *230*, 417.
(190) Admiraal, G.; van der Veer, J. L.; de Graaff, R. A. G.; den Hartog, J. H. J.; Reedijk, J. *J. Am. Chem. Soc.* **1987**, *109*, 592. Coordinates were provided by J. Reedijk.
(191) Caradonna, J. P. Ph.D. Dissertation, Columbia University, 1985.
(192) Sherman, S. E. Ph.D. Dissertation, Massachusetts Institute of Technology, 1987.
(193) Shakked, Z.; Rabinovich, D.; Kennard, O.; Cruse, W. B. T.; Salisbury, S. A.; Viswamitra, M. A. *J. Mol. Biol.* **1983**, *166*, 183.
(194) McCall, M.; Brown, T.; Kennard, O. *J. Mol. Biol.* **1981**, *149*, 761.
(195) Arnott, S.; Chandrasekaran, D. L.; Birdsall, A. G. W. L.; Ratcliff, R. L. *Nature (London)* **1980**, *283*, 743.
(196) Fratini, A. V.; Kopka, M. L.; Drew, H. R.; Dickerson, R. E. *J. Biol. Chem.* **1982**, *257*, 14686.
(197) Orbell, J. D.; Marzilli, L. G.; Kistenmacher, T. J. *J. Am. Chem. Soc.* **1981**, *103*, 5126.
(198) Gorenstein, D. G.; Findlay, J. B.; Momii, R. K.; Luxon, B. A.; Kar, D. *Biochemistry* **1976**, *15*, 3796.
(199) Haasnoot, C. A. G.; Altona, C. *Nucleic Acids Res.* **1979**, *6*, 1135.
(200) Hilbers, C. W. In *Biological Applications of Magnetic Resonance*; Schulman, R. G., Ed.; Academic: London, 1979; p 1.
(201) Sundquist, W. I.; Lippard, S. J.; Stollar, B. D. *Proc. Natl. Acad. Sci. U.S.A.*, in press.
(202) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; Marcelis, A. T. M.; van der Marel, G. A.; Rinkel, L. J.; Wille-Hazeleger, G.; Reedijk, J. *Eur. J. Biochem.* **1983**, *134*, 485.
(203) van der Veer, J. L.; Ligtvoet, G. J.; van den Elst, H.; Reedijk, J. *J. Am. Chem. Soc.* **1986**, *108*, 3860.
(204) den Hartog, J. H. J.; Altona, C.; van den Elst, H.; van der Marel, G. A.; Reedijk, J. *Inorg. Chem.* **1985**, *24*, 983.
(205) Marcelis, A. T. M.; den Hartog, J. H. J.; Reedijk, J. *J. Am. Chem. Soc.* **1982**, *104*, 2664.
(206) Gibson, D.; Lippard, S. J. *Inorg. Chem.* **1987**, *26*, 2275.
(207) Lepre, C. A.; Strothkamp, K. G.; Lippard, S. J. *Biochemistry*, in press.
(208) Polissiou, M.; Viet, M. T. P.; St. Jacques, M.; Theophanides, T. *Inorg. Chim. Acta* **1985**, *107*, 203.